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Problem-solving PhDs

ALTHOUGH the disillusionment of industry with at least some aspects of the present PhD system has been highlighted this year by the report of the House of Commons Expenditure Committee on postgraduate education, declarations on the need for change were made by academics and industrialists on the Swann Committee as long ago as 1968. That committee looked into the whole question of the flow of qualified personnel into employment and roundly stated that the universities should "experiment boldly" with the PhD and with the regulations governing its award.

Academics protest, quite rightly, that the PhD is not only a training in methods of research but also a means of preserving the continuity of university research—the aim of which is to generate new knowledge. That said, the question is whether too many 'conventional' PhDs are being produced. The fact is that of some 4,600 scientists graduating with higher degrees in 1971, some 1,200 took up employment outside teaching and lecturing, and although many of these had master's degrees a substantial number had doctorates. It seems logical therefore that some PhD students should carry out postgraduate work that will fit them for their eventual career in industry.

Changes along these lines have already been made by the Science Research Council (SRC). First, it has been running the so-called CASE scheme (Co-operative Awards in Science and Engineering). In 1972, 191 of these awards were made, out of a total of 2,220 new SRC research studentships. The SRC also offers postgraduate studentships to people who wish to do a postgraduate course after a spell in, for example, industry or teaching; 313 of these were taken up in 1972, for both research leading to a PhD and for advanced courses.

Like all collaborative schemes CASE has not been without its problems. The scheme requires joint supervision of the student by a member of the staff of the university concerned and a person from the collaborating institution, which following a relaxation in the rules may be a body like a hospital board or a local authority rather than specifically an industrial company. Trouble has arisen because the projects to be tackled have in some cases been badly or vaguely specified and often because of a lack of mutual understanding between the industrialist and the academic.

Yet another kind of doctorate that has emerged in the past few years is the interdisciplinary PhD. The idea arose from the discussions following the publication of the Swann report and involves a graduate, often, though not necessarily, a scientist, getting to grips with a real industrial problem which calls for an appreciation of economics, industrial relations and the like. The SRC and the Social Science Research Council

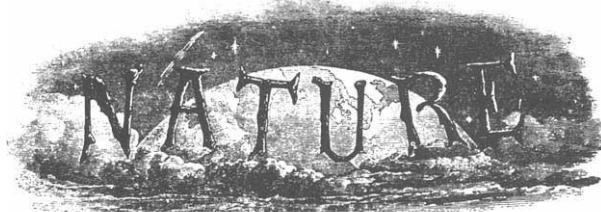
jointly fund almost 100 interdisciplinary research studentships, about 30 of which are held at the University of Aston.

The University of Aston is also in the throes of preparing for the advent of "total technology" PhD courses. These are specifically for engineers and have many of the characteristics of the interdisciplinary higher degree scheme but with more formal course work. What the research student is required to do is to solve real engineering problems in a real company. The University of Aston is well placed to take advantage of this because it already has the central organisation that is required if projects for students are to be thought out properly before the student arrives. The SRC awarded the first six total technology studentships last year.

That is all very well, but what scope is there for the pure scientist who wants to do a 'nonconventional' PhD yet wants something different from the CASE scheme. One answer is that there should be more scope for research done in an industrial environment to form the basis of a university PhD thesis. Unfortunately some universities take the view that they are not in the business of externally examining for a PhD. They might do well to re-examine such an isolationist attitude.

Some may say that to extend the PhD concept too much is to reduce the degree to a certificate of ability to solve problems. But does this matter? Provided that those who are really cut out to do research in the true sense of the word still have a reasonable chance to do it the appearance of more doctors of problem solving (which is all some 'conventional' PhDs are at the moment anyway) seems to be much more of a benefit than a threat.

100 years ago



AN extract from a letter by Mr. Dunn, the geologist, now on a special exploring expedition to the Transvaal, published in the *Cape Argus* of May 5, gives a description of a thunder and hail-storm which he experienced at Pietermaritzburg, on April 17:—
 "Hail-stones, liberally mingled with great masses of ice of very irregular forms, poured down with great violence. The hail-stones were seldom less than 1 in. in diameter; the average was from 1½ in. to 2 in. in diameter. These were of very regular spherical form, and consisted of a nucleus of white snow, with an envelope of hard transparent ice. Sometimes they presented, when broken through, a concentric arrangement of zones, alternately white and opaque and transparent. The irregular masses were formed of a nucleus generally longer in one direction than the others, from 2 in. to 4 in. in diameter; projecting all over were stalactites, each one about the thickness of a little finger, and presenting, when broken across, an agate-like structure, as though segregation had built them up. Of these masses I weighed a few with the following results:—Three weighed over 8 oz., two over 6 oz., and one over 4 oz. The mischief done will not be covered by 2,000l. or anything like that sum."

From *Nature*, 10, 115, June 11, 1874.



Russia's curtained window on the west

In the 250th anniversary year of the Russian Academy of Sciences, Vera Rich examines the rôle the Academy has played in international science.

It can hardly be doubted that, in founding his Imperial Academy of Sciences, Peter the Great intended it to form one of the cornerstones of his Westernising policy—a consummation at the highest intellectual level of the European outlook that had driven him, earlier in his reign, to cut off his subjects' beards and to impose the death penalty on courtiers found sleeping with their boots on.

Yet Peter, who was personally curious about all branches of science and technology from the theory of navigation to do-it-yourself dentistry, having, after more than 20 years' cogitation, finally signed the decree "establishing" the Academy, on January 28, 1764, did not live to attend its opening. Exactly a year later, he died, succumbing to a chill caught, not in the pursuit of his innovations, but as a result of attending the traditional Epiphany Eve blessing of the waters! It was left to his widow and successor, the erstwhile illiterate Livonian peasant-girl, who reigned as Catherine I, to bring into being Peter's Academy, and it was on her nameday, December 27, 1725, that the inaugural meeting was held.

Although he is reputed never to have opened a book (and it must be said in passing that the improbably tall stories surrounding his name are more than legion), Peter seems to have been giving serious thought to the foundation of some eminent scientific body to grace his projected new capital and "window on the west" as early as the 1690s. During his visit to England in 1698, he visited the Royal Society (January 27), Greenwich Observatory (March 9) and Oxford (April 8) where he was presented with a number of mathematical books.

Back in Russia, he founded a Naval Academy, Artillery, Engineering and Medical Schools, and Russia's first national museum, the *Kunstkamera*, in his new capital of St Petersburg, together with several printing presses using the simplified typography, which he had introduced in 1708. He also began a prolonged correspondence with Leibniz, who had organised the Berlin Academy of Sciences in 1700, on the possibility of establishing a similar



The Academy's Moscow headquarters.

institution in St Petersburg. Although neither Peter nor Leibniz were to see the Academy come to fruition, nevertheless, in 1725 the first meeting finally occurred, and with a membership mainly of foreign, especially German savants.

This foreign, or rather, international, membership, persisted through the first difficult years of the Academy's existence (it had no official constitution until 1747, nor any established source of income). Prominent Academicians of that time include such eminent names as Nicholas (II) and Daniel Bernoulli (elected 1725), Leonard Euler (1727) and Georg Wilhelm Steller (of 'seacow' fame) (1737). It was from Euler's time at the Academy that the famous problem of the 'seven bridges'—one of the early landmarks of topology—dates. In spite of this source of inspiration, Euler found the atmosphere of St Petersburg unconducive to the free exchange of ideas, and in 1741 he accepted an invitation from Frederick the Great to join the Berlin Academy. (Asked by the Dowager Queen why he could only, apparently, speak in monosyllables, Euler is reported to have replied that "I've just come from a country where, if you speak, you're hanged!") In 1785, however, on the invitation of Catherine II, Euler returned to St Petersburg, where, in the intervals of research, he was expected to produce such diversions for the court as a formal mathematical refutation of the 'atheistic' views of the visiting French philosopher Diderot. (Euler's tongue-in-cheek "proof": "Sir, $(a+b)^n/n=x$, hence God exists;

reply!", astounded the court, discomfited the unmathematical Diderot—and is reported to have found a place in current theological treatise!)

In spite of such Imperial digressions, the Academy at last settled down to producing its own scientists and scholars. Among numerous lesser luminaries, one finds names such as Lomonosov (elected 1741), Lenz (1828), Ostrogradskii (1828) and Krylov (of the *Fables* (1841).

There was still, however, a strong international atmosphere, reflected specially in the honorary members, who included Réaumur (1737), Voltaire (1778), Linné (1754), Boscovitch (1759), d'Alembert (1764), Diderot (a reward, maybe for valour?) (1773), Lagrange (1776), Bode (1794) and Kant (1794). British honorary members included such well-known figures as Maskelyne (1776), Priestley (1780), Sir Joseph Banks (1780), Malthus (1826), Sir William Parry (1826), Sir Humphrey Davy (1826) and Faraday (1830). In the eighteenth century, the United States was represented by honorary members Benjamin Franklin (1789) and John Churchman of Philadelphia (1795).

Yet in spite of its international beginnings, its use of Latin, French or German as its medium of publication (the *Comptes Rendus* of the Academy was still appearing in French in the 1940s), and the continuing election of foreign scientists as honorary members, the international contacts of the Academy did not proceed smoothly and unbrokenly. The early years of the nine-

Peter the Great: intended the Academy to form one of the cornerstones of his Westernising policy—a consummation at the highest intellectual level of the European outlook that had driven him to cut off his subjects' beards and to impose the death penalty on courtiers found sleeping with their boots on.

teenth century showed the Academy fallen on hard times. This was partly as a result of the foundation of new universities, so that the Academy lost a certain amount of prestige, being no longer the *ions et origo* of all learning in Russia, partly this was due to the conditions of the Napoleonic wars. Further, under Paul I (1796–1801), the receipt of literature from abroad was forbidden and international contacts were broken off. The membership of the Academy was reduced to half its original strength—and a large proportion of the remaining members were superannuated veterans, no longer able to attend meetings!

Paul's successor, Alexander I, was keenly interested in education, founding Universities (or reconstituting those, Vilna and Dorpat, which he had gained in recent territorial acquisitions). But although the Academy gradually regained its old strength and vigour, the vexed question of censorship remained. Knowledge might, and did, flow out of Russia—it was considerably more difficult for it to flow, or even to trickle in. The nineteenth century witnessed a running battle between the Academy and Universities on the one hand and the Government on the other, on the right of learned bodies to import foreign literature, especially periodical literature, without inspection by the censors. (It should be noted that censorship was, at times, ludicrously rigid; on one notable occasion a mathematical treatise was refused publication since it was feared that the "... of the formulae for infinite series denoted a lacuna into which some revolutionary or otherwise treasonable matter would be inserted by dissident minds.) Although from the beginning, the Academy and Universities had been able to import scientific works (indeed, without them, no learned institution would have ever been able to begin functioning), the dispute arose out of the definition of "scientific". The new constitution of Vilna University (1803) implies that the Universities are to censor themselves. The decree on censorship of 1828 permitted the Universities and the Academy to "receive books without inspection by the censorship", but they are obliged "every time to inform the censorship committee for foreign books what the books are, how many, and when and by what route they were sent".

Although even the natural sciences (with their suspect symbolism) were not



exempt from import difficulties, the problem was particularly pressing with the 'philosophers', who needed to read current political journals. The restrictions seem to have been imposed somewhat intermittently, according to the prevailing political atmosphere. Thus in the 1840s for example, the Academy was able to receive the *Allgemeine Zeitung* and *Berliner Nachrichten* fairly freely. A decree of 1860, however, imposes somewhat narrow limitations: "The Imperial Academy of Sciences, the Pulkova Observatory and the Universities may enjoy the right to obtain through the post from abroad books and periodical literature of academic content in an open wrapper, without extension of this right to members of the Academy and Universities." And since the opinion of the censorship on what was, and was not academic, was extremely arbitrary, one may find in these restrictions at least a partial explanation of the many claims that Russian scientists have discovered or elaborated some principle "independently" of work done abroad. Although most of the more fantastic claims disappeared with the excesses of the personality cult, one still finds from time to time references, for example, *Pravda*, June 12, 1973, to Popov as the inventor of radio. Such parallelism may be attributable, at least in part, to these

censorship difficulties—a Russian working in a given field might well have been completely unaware of what his opposite numbers abroad were doing. Yet, conversely, certain eminent nineteenth century Russian scholars were never to become Academicians, Lobachevskii spent his life in the backwater of Kazan' and Mendeleeev was refused membership on account of his dangerously 'liberal' views. One way and another, the nineteenth century Academy lost much by governmental intervention.

From the leisured days of the beginnings of the Academy (one eighteenth century silhouette shows an open air meeting reminiscent of a *fête champêtre* rather than a learned discourse) to the earnest days of post-Revolutionary Petrograd seems a far cry, yet, according to an official report of 1927, one of the first things that the new régime did was to restore the old international contacts. "In the last ten years, the international relations of the Academy have taken the following forms: (1) participation of the Academy in permanent international unions and associations of general scientific interest or of special disciplines; (2) joint scientific enterprises, such as expeditions, publications, etc.; (3) participation of the Academy in International and other meetings and congresses convened

abroad, and in the anniversaries of learned societies, scientific institutions or individual scholars; (4) scientific missions abroad, including the permanent work of our scholars in laboratories of other countries; (5) rational and consistent exchange of scientific publications; (6) exchange of collections and the dispatch abroad, on a basis of reciprocity, of collections, manuscripts, and rare editions; (7) awarding of prizes and honorary titles to foreign scholars and mutual invitations to lecture at higher education institutions or to take part in conferences on particular occasions and, finally, rational organisation of the acquisition abroad of publications, instruments, apparatus and all other scientific material, necessary for the Academy and its institutions".

To what extent this excellent programme was implemented it is difficult to judge—official reports of this kind always have a tendency to overstate achievements. In any case, the picture was due to change. With the Stalinist purges of the 1930s, all contact with the West became not only discouraged—they were positively dangerous for those involved. Scientists who had trained abroad (under the plans so recently praised), were particularly vulnerable. In the Academies of the Union Republics (now virtually satellites of the All-Union Academy) where the accusation of 'nationalism' or 'separatism' was always possible, the situation was particularly tense. The Kiev Academy, for example, had no less than 13 Secretaries between 1921 and 1938, all of whom were, in turn, arrested. The election of foreign honorary members and corresponding members to the Academy came to a sudden halt. In the immediate post-revolutionary period, no foreign members were elected: then one member was elected in 1922, 18 in 1924 (including Frederick Soddy), one in 1925, one in 1926, five in 1927, four in 1928, nine in 1929, three in 1930, two in 1931, six in 1932, one in 1933, and in 1934, the year in which the Academy formally moved its headquarters (with 250 wagonloads of equipment and more than 300 scientists) to Moscow, time was still found to elect five new foreign members (of whom Max Born was one).

Then comes a complete gap. No further foreign honorary members or corresponding members were elected until 1942, when five new names (including J. B. S. Haldane) appear in the records, presumably as a symbol of Soviet friendship towards the Allies.

As for the work produced by the Academy during the 1930s, in spite of the new site, increased subsidies, the foundation of new institutes, and all other material government help that a learned institution might well desire, the growth of what was later to be

known as the 'personality cult', was not conducive to sustained and concentrated research. As Robert Conquest has observed, although the man in the street could, in conditions of peril, stop talking, the professors and academicians had to go on lecturing! With the constant attrition due to the disappearance of those whose views failed to coincide with the current official line, and the occasional endorsement of a new theory from Party headquarters, Soviet science was not noted for the free dissemination of ideas. It became inward-looking, and self-congratulatory. When the 220th anniversary of the Academy was celebrated in 1945 (the 'Catherine' date being chosen, since on the 'Peter' date, the Soviet Union was fully involved with her war effort) we find in the proceedings of the occasion that the history of science has been considerably revised!

In aerodynamics, Mendelev anticipated Prandtl's views on hydrodynamic friction by some 30 years, and a certain Rykachev in 1871 forestalled Eiffel's experiments on the lift of an airscrew by 40 years. The Danish Bering was no longer the discoverer of the Strait which bears his name—he merely confirmed the discovery made by "the Cossack Deinev" in 1648. The forces of "chemical constitution" (the nature of the chemical bond) were investigated by Butlerov in 1862—although it is somewhat naively observed that as with many other Russian scientists, "his work was not always duly appreciated abroad!"

In the 1950s the Academy made a determined effort to free itself from the tight controls which demanded an immediate economic outcome of all projects, and, in spite of conventional lip-service to successive five-year plans, does manage to achieve a considerable

amount of basic research that has little immediate connection with the needs of the economy.

On the international scene, however, all is not entirely well. International co-operation does take place—but with an increased emphasis on co-operation within the Comecon block or on aid to the developing science of the third world. Specific agreements for co-operation between the Soviet Union and a specific Western country on a specific project or set of projects only underline the lack of a general atmosphere of free exchange. Soviet scientific journals flow out freely to the West (and agreements have been made for the production abroad of cover-to-cover translations which appear within a few months of the Russian originals). But western journals entering the Soviet Union do so in single copies, which are then photocopied and distributed to academic institutions with any doubtful matter replaced by back-material of a more innocuous nature (for example, advertisements). The campaign of September 1973 against Academician Sakharov stated that "To be a Soviet scientist means to be a patriot"—one must not bite the Party hand that subsidises one's research, by advocating too close a co-operation with the west. The position of Jewish scientists who wish to emigrate to Israel who are refused a visa to emigrate, but at the same time dismissed from their posts so that they can no longer exercise the talents which the state is supposedly conserving for its own use has raised new and bitter feelings abroad.

The inaugural address of 1725 spoke of bringing Russia into the world scientific community. But, no reference was made to the fact that the Royal Society, so admired by Peter, specifically excluded all considerations of the religious or political views of its members—nor, would it seem, has the Academy yet learned, in the 250 years of its existence, to view a scholar's scientific achievements apart from his personal philosophy. The cancellation of the international celebrations of the anniversary of the Academy, and its replacements by a programme of popular scientific lectures to the grass-roots of Soviet Society—the workers and peasants, is more, therefore, than a gesture of pique over recent criticisms abroad of the restrictions and lack of academic freedom imposed on certain intellectuals in the Soviet Union—rather is it a repetition once more, of a pattern of isolationism which runs through the history of the Academy, a firm closing, yet again, of what was to have been one of the most prominent casements of Peter's "window on the west".

Charter of the Academy.

PRAEFATIO.

*Leges, quibus Academicam suam AUGUSTA PRO-
TECTORIX regit,
inter cetera salutariter statuta, etiam id officii Academicis injungunt, ut, pro excolendis communi consilio & cura scientiis, bina singulis hebdomadis vice omnes conveniant; alteri, ut sine destinationes, sine commentationes suas Sociorum iudiciis exponant; alteri, ut communicatis invicem sententiis, si quid de argumento disquisitioni exposito utile*

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international news

Canada's reply to Indian nuclear explosion

David Spurgeon, Ottawa

CANADA has responded quickly and firmly to India's explosion of an underground nuclear device. External Affairs Minister Mitchell Sharp announced four days after the weekend's test that Canada has suspended all nuclear assistance and cooperation and recalled Atomic Energy of Canada Limited's resident representative.

Mr Sharp said that the Canadian government is very disturbed by the tests, in which a device of approximately 15 kilotons yield was reported to have been exploded at a depth of 100 metres. "For all intents and purposes", he told a press conference, "India now has developed the capability of producing a nuclear weapon".

Canada's concern has resulted from the likelihood that the plutonium used in the explosive device came from a reactor named CIRRUS that was a gift from Canada. Canada has also designed and helped to build and equip two electric power reactors at Rajasthan (Rapp I and Rapp II), and most of India's nuclear personnel have been trained in Canada.

Canada has long been fearful of India's nuclear intentions. Copies of correspondence between Prime Minister Pierre Trudeau and India's Prime Minister Indira Gandhi in October 1971, which were distributed at the press conference, reveal Canada's concern at that time for the prevention of proliferation of nuclear weapons.

"The use of Canadian supplied material, equipment and facilities in India, that is, at CIRRUS, Rapp I or Rapp II or fissile material from these reactors, for the development of a nuclear explosive device would inevitably call on our part for a reassessment of our nuclear cooperation arrangements with India, a position we would take with any other non-nuclear weapons state with which we had cooperation arrangements in the nuclear field", Mr Trudeau warned.

"My government", replied Mrs Gandhi, "reiterates its commitment to the provisions contained in the nuclear cooperation agreements between India

and Canada to which your government is also committed. Our two governments have acted in conformity with these arrangements for the past several years. The obligations undertaken by our two governments are mutual and they cannot be unilaterally varied".

What appears to have let the Indians off the hook, at least in their own minds, is their interpretation of the underground device they exploded as a "peaceful use" of nuclear energy. This interpretation has never been shared by Canada, but it seems to have been India's all along. Canada signed the Nonproliferation Treaty but India refused on the grounds that it was "discriminatory".

Canada did win inspection provisions for the two Rapp power reactors by the International Atomic Energy Authority but there were no such provisions for the CIRRUS reactor. CIRRUS seems the most likely source of plutonium for the device. For other reasons: AECL officials say that the one Rapp reactor now running has not been operating long enough to produce sufficient plutonium for an explosive device. The Rapp reactors are of the Candu type, fuelled with natural uranium and they are not ideal for producing weapons grade material in any case.

The CIRRUS was initially fuelled by Canada but subsequent fuel was manufactured by the Indians themselves, according to Canadian officials. The Indian fuel was not subject to inspection. Thus the plutonium for the device could have come from the Indian fuel and have been separated out in India's reprocessing plant, which has been operating since 1964. In fact, press reports in India were said by a spokesman in Canada's Department of External Affairs to have quoted Indian officials as saying that this was the case.

Asked for his personal reaction to news of the explosion, J. L. Gray, President of AECL, said quite frankly "I was really quite surprised and very disappointed". Gray suggested that halting Canada's nuclear assistance at this point will have little effect on India's nuclear power programme. "It might delay them but it won't stop their programme," he said. One of the Canadian-built Rapp reactors is already running, the other is nearly finished and India is building four more of its own.

In future Canada will try to assure that there is no ambiguity about the

meaning of the phrase "peaceful uses" in agreements with other countries. But it is not clear how that will be accomplished for Mr Trudeau's 1971 letter to Mrs Gandhi seemed clear enough. Mr Sharp said that Canada "made it clear in international discussions and in bilateral exchanges with India that the creation of a nuclear explosion for so-called peaceful purposes could not be considered as a peaceful purpose within the meaning of our cooperative arrangements".

India's action has also brought up the question of Canada's aid programme to that country in general. Mr Sharp said that Canada is conscious of the very large costs involved in the development of nuclear energy for peaceful purposes and appreciates the substantial resources needed for the development of explosive devices. "Canada does not intend to share the burden of relieving such costs" he said. Thus the Canadian government is not prepared to agree to any roll over of India's commercial debt to Canada which is largely related to her nuclear energy programme.

Apart from the danger of nuclear weapons proliferation, many Canadian officials have found it hard to understand the rationale of putting a high priority on an expensive nuclear device which has a questionable utility while such vital national problems as assuring an adequate food supply continue to go unresolved. Indian spokesmen said the device was needed to aid in developing new energy sources, but understood nuclear devices have not been notably successful in such uses, even in the United States.

Oil from Russia?

from our Soviet Correspondent

SPEAKING in London recently, Academician Vladimir A. Kirilin, Deputy Chairman of the Council of Ministers of the USSR and Chairman of its State Committee for Science and Technology, called for cooperation between Britain and the USSR over energy matters—both in nuclear energy and oil production.

The occasion was the third meeting of the Permanent UK/USSR Intergovernmental Commission for Co-operation in the fields of Applied Science, Technology, Trade and Economic Relations. Mr Peter Shore, Secretary of State for Trade led the United Kingdom delegation and pre-

sided; Academician Kirilin headed the Soviet delegation.

The meeting paid considerable attention to ways of implementing the recently signed (May 6, 1974) Ten Year Agreement on the Development of Economic, Scientific, Technological and Industrial Cooperation, studying, in particular, the cooperation in major technological projects on a compensation basis and the necessity for flexibility in planning individual cooperative ventures.

It was recommended that further exchanges should take place in the fields of crystal structure and macromolecules, astronomy, phase synchrotron radiation, plasma physics, hydrostatic extrusion, corrosion and tribology.

At the press conference after the meeting, Academician Kirilin said that cooperation in the field of breeder reactors "in which both countries have had notable achievements" could considerably reduce the cost of energy production.

As to oil production, he said that talks have begun with British Petroleum, to be continued in Moscow next month, to outline a possible scheme by which the United Kingdom would provide the plant and that in return the Soviet Union would deliver oil for BP "for we do not expect at the moment, the UK will deliver oil to the Soviet Union!". This, however, he stressed, is still in the planning stage, and a number of possible options for co-operation in this field lie open.

Speaking generally, Academician Kirilin stressed that the current round of talks and, indeed, the 10-year agreement could only bear their full fruit in an atmosphere of detente and co-operation, which, he averred was a fundamental tenet of Soviet foreign policy. This raised a number of questions about the rights of scientists to emigrate, and what proportion of the goods to be exchanged had originated in Labour Camps (the questioner was doubtless thinking of *The First Circle*). Academician Kirilin replied that there were no labour camps in the Soviet Union, and that because of semantic difficulties, this question could not be profitably discussed, that "almost all" scientists who wished to emigrate could do so—the exceptions being those concerned with national security, and that as for the Panovs (who as dancers could hardly fall into that category), he personally had never heard of them until he came to London, but he was sure their fate "would be dealt with fairly". At which point, in the cause of detente and cooperation, Mr Shore intervened and brought the discussion back to the safer channels of mutual economic benefits and exchange.

After the conclusion of the official talks, the Soviet delegation began a

programme of visits to laboratories and research establishments with which possible cooperation was envisaged. The first visit on the programme was to the fusion laboratories at Culham, where Academician Kirilin presented a paper read on his behalf by Dr John Chubb, on the present and future energy situation in the Soviet Union. His data seemed perhaps, a little on the optimistic side—fast-neutron reactors a practical possibility by 1985, and it was interesting to note that in spite of the importance which he places on co-operation in thermonuclear research as a source of cheap power in the future, he stressed the immediate emphasis in the Soviet Union on the coal industry, and recommended a rethinking of the advantages of coal to the West!

Archaeology in crisis

from a Correspondent

A BASIC reorganisation of archaeological excavation in Britain is heralded in a recent press release from the Department of the Environment. It scarcely hints, however, at the underlying problems created by the increase in rescue archaeology, and by the apparent failure of the Department of Education and Science to respond to the current archaeological crisis with the awareness shown by its sister Department.

Mr Crosland's announcement promises an increased grant of £1,063,000 for rescue excavation and post-excavation work in Great Britain during 1974–5. When compared with the grant of £440,000 for 1972–3, and £813,000 for 1973–74, this shows a continuing awareness of the widespread destruction which urban development and mechanised farming are wreaking upon what is left of Britain's past. For the evidence can be rescued only now, before it is gone for ever.

The accelerated growth of rescue archaeology in this country has created its own practical problems, however. There is a shortage of skilled archaeological fieldworkers for the senior positions, and especially of trained diggers—the equivalent, in a sense, of laboratory technicians in the natural sciences. Even so, there are now many hundreds of these diggers, working all the year round, replacing at least in part the amateur archaeologists who until a decade ago made up the entire work force on any summer dig. The emergence of what is in effect a new profession—of full-time digging archaeologists—requires some coordination in terms and conditions of employment, and trade union membership is now under active discussion (*The Times*, May 25, 1974). The formation of some sort of Association of Professional Archaeologists has now been proposed,

which could propose and promote some unified career structure, serving the varied interests of archaeologists in museums, the universities, the civil service and the local authorities as well as the full-time diggers.

These logistic questions are less important, however, than the crucial one of using the annual rescue budget of £1 million to good effect. Hitherto this has been entirely the responsibility of the Ancient Monuments Inspectorate of the Department of the Environment—and its very title recalls that until a decade ago the Inspectorate's principal task was the maintenance of the ancient buildings and archaeological sites under its guardianship, and the protection from destruction of other sites listed as scheduled monuments. The explosive growth of rescue excavation, financed by the Department of the Environment, has put heavy new responsibilities upon its archaeological personnel.

The second, and ultimately more important part of the Minister's announcement takes account of this by setting up a number of advisory committees. The first is a new national advisory committee (a sub-committee of the Ancient Monuments Board, which advises the Secretary of State on the scheduling of Ancient Monuments) to advise on the priorities of archaeological excavation in England. In addition 13 advisory committees are to be set up on a regional basis to advise on local priorities for rescue excavation and archaeological survey where sites are threatened.

It remains to be seen how this structure of regional committees and a national committee will operate (and there has been immediate criticism that the regional committees are not to have direct representation on the national committee). But at least it goes some way to meet a fundamental point, which the urgency of rescue excavation and the expenditure of public money upon it both at national and local level, has sometimes served to obscure. The only purpose of rescue excavation, where the site itself is by definition doomed, is the provision of information about the past. All rescue excavation is research, aimed at providing new information to enlarge our knowledge and understanding of the past. Yet hitherto, as both the Council for British Archaeology, and Rescue, The Trust for British Archaeology have pointed out, there has been no coherent research strategy of any kind in this country. Sites have been dug simply because they were threatened with destruction. But while this is a necessary condition for the expenditure of public funds, it is certainly not a sufficient one, for even the increased funds allow for the complete excavation of only a tiny fraction of the sites threatened. In general, in-

deed, there is too much hasty digging and not enough thinking, resulting in the production of large quantities of data without any clear idea of just what problems it is supposed to solve. Moreover the total failure of publication to keep up with the increase in excavation has recently been stressed (Addyman, *Rescue News*, 6, 1; 1974), nor can all the publications which appear be rated as significant contributions to our knowledge of the past. These are problems which the new advisory committees must now tackle urgently if the increase in rescue excavation is to be in any way effective in counteracting the loss of evidence by development and destruction. The task now is to make rescue research more effective in terms of significant results and their rapid publication.

And it is here that the Department of Education and Science has so far failed for, unlike the Department of

the Environment, it actually has a formal public commitment to scientific research. Now, archaeological excavation under the often hasty conditions implied in rescue work, does not offer the ideal laboratory for the development of new techniques and the investigation of entirely new approaches to the past which are essential for real progress. Yet archaeological research is considered as outside the field of interest of each of the three research councils (SRC, SSRC and NERC) which might be expected to support it, to the extent that the only way an archaeological research project can find support from them is to masquerade as something else and lodge itself in a department of physics or geology. The British Academy, to be sure, makes a number of small grants, but these are distributed over the whole field of the humanities. Some time ago the British Academy and the Royal Society held joint dis-

cussions on this curious state of affairs. It is believed that they resolved jointly to approach the Department of Education and Science with a view to the institution of some body, analogous to the SRC or to NERC, by which scientific research in archaeology could be supported. But so far nothing more has been heard. So we have the paradox that rescue excavation is supported by the government on an increased scale, reflecting real awareness of the archaeological crisis, while the further scientific research which should exploit this great opportunity by analysing existing data and by developing new techniques, is simply not taking place. Archaeological research in Britain is thus in a state of considerable confusion. Mr Crosland's advisory committees will have to work long and think clearly if the funds for rescue excavation are to yield commensurate scientific and historical rewards.

The future of National Parks

Peter J. Smith

ACCORDING to the late John Dower, a National Park in the British context was to be "an extensive area of beautiful and relatively wild country in which . . . (a) the characteristic landscape beauty is strictly preserved, (b) access and facilities for public open-air enjoyment are amply provided, (c) wildlife and buildings and places of architectural and historic interest are suitably protected, while (d) established farming use is effectively maintained". The precise wording of the National Parks and Access to the Countryside Act 1949, under which the ten National Parks of England and Wales were later created, was slightly different that they were to be designated areas in which an attempt would be made by careful control to avoid radical change in either natural or man-made characteristics whilst encouraging greater recreational use.

Twenty-five years later the result, now examined by a committee under Lord Sandford (*Report of the National Park Policies Review Committee*, HMSO, London, 1974), can be seen to be mixed. There is no doubt that the National Parks have managed to maintain some distinction and are generally held in high public regard, but at the same time they have been eroded to a much greater extent than their creators envisaged or would have desired. Part of the conflict between the ideal and the reality has arisen because, rightly or wrongly, the 1949 Act made no attempt to change the ownership of the land within the designated areas, and so total responsibility for each park has never been in the hands of a single



administration. This lack of complete control by park authorities has been particularly unfortunate where it relates to the use of land previously established for farming.

As Sandford points out, the scenic quality of the man-made part of the environment in National Parks is due primarily to the grazing of sheep and cattle, which maintains the characteristic open landscapes. But large scale afforestation of open land with alien conifers has taken place as a result of national economic decisions—a development which park authorities have been largely powerless to prevent, and heather moorland and other rough grazing have been converted to more productive pasture altering the open character of the country and impeding access. The political difficulty is that in the last resort the park authorities have little power over the activities of private landlords. Sandford therefore recom-

The Scafells across Wastwater: inviolate?

mends that where mutual agreement on land use proves impossible, the authorities should have the power of compulsory purchase, and that afforestation in particular should be subject to planning controls.

As for development, parks have generally been able to avoid excessive residential and small-scale commercial development but there has been what many people take to be a deplorable amount of large-scale development, generally under pressure from central government and supposedly "in the national interest". Sandford's view is that because the National Parks account for 9% (over 3.3 million acres) of the area of England and Wales, and contain important mineral and water resources, it would be unrealistic to hold them inviolate against such development. At the same time, the

report acknowledges that "in the past amenity values appear to have been set aside too easily", and suggests that in future there should be a strong presumption against development which conflicts with park purposes.

Some of the parks' more obvious difficulties have arisen from the inherent conflict between preservation, on the one hand, and public enjoyment of the preserved facilities, on the other; the ever-increasing number of visitors has begun to threaten the very characteristics which attract the visitors in the first place. Road alterations have been carried out with no overall scheme in mind and in ways more appropriate to the urban than the rural environment; cars, coaches and caravans have been allowed to penetrate 'sensitive' areas; and roadside walls and verges have suffered from over-heavy vehicles.

The general problem here as Sandford sees it is that both park authorities and the government have been slow to

react to changing circumstances; they have failed to come to terms with the fact that, although the walkers and climbers in the forefront of thinking during the 1940s are now more numerous than ever, the drivers have multiplied at an even higher rate. What, specifically, should be done? The Sandford recipe includes the routing of new trunk roads around the National Parks, the abandonment of any attempt to maintain uncongested traffic flow within the parks by means of new road development, refusal to permit alterations in existing road width or alignment, encouragement of peripheral parking linked to public transport inside the parks, and the resiting and screening of prominent caravan sites. Of course, it is one thing to identify sensible solutions, but quite another to carry them out—and the Sandford committee goes little into detailed implementation. On the other hand, there can be no doubt that the basic principle is sound

and worth restating—that where the preservation and enhancement of beauty conflict acutely with the promotion of public enjoyment, the former must prevail.

Finally, the report suggests that there should be designated "national heritage areas" in which "the conservation of environmental qualities would be the supreme objective . . . taking precedence over all others". There would be no settlement larger than a hamlet, no industrial development, no quarrying or mining, no reservoirs or defence works, no main roads or railways, no facilities for visitors, and visitor access would be limited to foot, bicycle and horse. This is the one genuinely radical suggestion in the whole report, although it is actually a minority proposal from four members of the committee. It deserves the widest debate, for this may be the last available opportunity for conserving "areas of the highest environmental quality".

correspondence

Chilean symposium

SIR,—We are scientists who firmly hold political opinions which differ from one to another. We are not concerned to try and understand these differences. We are, however, convinced that every scientist (like every nonscientist, indeed) has the right to hold what ideas he likes and to maintain them within his own country in the manner which he deems best.

We are similarly convinced that all political matters should be excluded from international scientific associations. Officialdom, no more than the organisers of, or the participants in international meetings, should neither impose nor allow any mixing of science and politics.

This is why we object to the following phrase "the re-establishment of social peace and internal order guarantees and secures to carry out this scientific event" in the invitation emanating from the Chilean section of the International Association of Volcanology and Chemistry of the Earth's Interior to attend a symposium in Santiago.

We cannot accept that a regime which has covered its large and unhappy country with blood, and which still continues to permit torture and political assassinations, should be permitted to include such a phrase in its circular of invitation to foreign scientists.

Needless to say, we do not propose to participate in this symposium and are of the opinion that a similar attitude should be adopted by any volcanologist sensible of the terrible Chilean tragedy, since his very presence there might tacitly imply tolerance from the human point of view.

Yours faithfully,
FR. BARBERI

Pisa

E. LOCARDI

Rome

G. MARINELLI

Pisa

M. MITTEMERGER

Rome

A. RITTMANN

Catania

H. TAZIEFF

Paris

J. VARET

Paris

Spilt milk

SIR,—John Hall rightly adduces several arguments against the change from breast feeding to bottle feeding which is apparent in less developed countries. But his description of the character of the "Baby Killer", bottle feeding, is incomplete as it does not mention an effect of bottle feeding that will probably kill even more

babies than the associated infections, malnutrition and poverty do. Lactation postpones postpartum ovulation and therefore contributes to birth spacing. It has been shown that prolonged lactation is indeed an important factor in controlling population growth. Populations in less developed countries that are changing from breast feeding to bottle feeding are likely to be insufficiently familiar with other means of family planning to compensate for this growth mechanism. I know of a case where all the female patients in a rural East African hospital were given a bra as a Christmas present, though family planning programmes in that area had not yet yielded any obvious result and the women concerned had hardly ever shown interest in bras. Whether due to well-intentioned grafting of our breast-centred culture into less developed cultures or due to deliberate promotion of dairy and bra industries, such behaviour is likely to add to baby killing population pressures. We should be aware of such incipient trends disturbing the balance between mortality and birth rates in less developed countries in addition to those already caused by Western medicine and technology.

Yours faithfully,
J. WIND

*Institute of Human Genetics,
Free University of Amsterdam.*

news and views

Gene expression in development

DIFFERENTIAL gene transcription is often put forward as the major source of control for protein synthesis during differentiation and development. But there are other possibilities such as nuclear processing and selection of messenger RNA for transport as well as various forms of translational control. It is clear that gene expression has a quantitative and a qualitative aspect since highly specialised cells such as reticulocytes, lymphocytes, silk gland and lens cells all produce a large amount of a few polypeptide chains and a small amount of a much larger number of proteins. Greater rates of transcription from genes appropriate to the end cell line may account for the quantitative differences in protein synthesis observed in these cells, although other possibilities such as differential mRNA stability, for which there is some evidence (Kafatos, *Fifth Karolinska Symposium*, 319; 1972) are not rigorously excluded.

Presumably gene expression also has a qualitative aspect but examples are harder to find and interpret since the answers obtained depend on the level of discrimination set by the techniques used in the analysis. Nucleic acid hybridisation procedures have recently provided evidence in a variety of systems for qualitative differences between RNAs extracted from different adult organs of the mouse (Brown and Church, *Devl Biol.*, **29**, 73; 1972); Grouse, Chilton and McCarthy, *Biochemistry*, **11**, 798; 1972) and different developmental stages of the slime mould *Dictyostelium* (Firtel, *J. molec. Biol.*, **66**, 363; 1972). These workers have used purified labelled unique DNA sequences in nucleic acid hybridisation reactions with RNA in excess to investigate the proportion of unique DNA sequences represented by RNA transcripts in any given RNA preparation. Either total cell or total nuclear RNA preparations have been used to drive the hybridisation reactions. To compare two RNA preparations they are hybridised separately and together under identical conditions and the amount of labelled unique DNA found in RNA/DNA hybrids can then be determined. From the results it is possible to compute the sequences common to both and also those present in one but not the other. For instance, in *Dictyostelium*, Firtel has shown that about 20% of the genome is represented in RNA at all stages of development and about 37% consists of sequences expressed at particular developmental stages. These studies provide evidence for qualitative changes in gene transcription during developmental and differentiation, but do not throw light on the question of qualitative differences between nuclear and cytoplasmic unique gene transcripts.

Two recent articles (Smith, Hough, Chamberlin and Davidson, *J. molec. Biol.*, **85**, 103; 1974; Galau, Britten and Davidson, *Cell*, **2**, 41; 1974) suggest in a developmental situation that the total number of diverse unique DNA sequence transcripts in nuclear RNA is ten times greater than in mRNA from polysomes. Using sea urchin embryos at the gastrula stage of development Smith *et al.* isolated heterogeneous nuclear RNA (hnRNA) labelled by a 10 min pulse of ^3H -uridine. The hnRNA was fractionated in non-denaturing sucrose gradients to give three fractions sedimenting less than 23S, between 23 and 40S and greater

than 40S. The greater than 40S fraction was used for hybridisation experiments with unlabelled sheared total DNA in excess under various conditions.

First the amount of hnRNA hybridising to DNA by *Cot* 40 was determined at two DNA/RNA ratios. These conditions represent an assay for repetitive sequence transcripts since by *Cot* 40 75% of repetitive DNA sequences but less than 5% of single copy DNA should have reannealed. About 25% of total ^3H -labelled hnRNA is recovered in hybrids separated from non-hybridised RNA on urea hydroxyapatite columns. Treatment with ribonuclease before recovery of hybrids reduces this value to 5–8% of total ^3H -hnRNA, suggesting that one-fifth to one-third of the hnRNA hybridised by *Cot* 40 is in DNA/RNA duplex and the rest is in unhybridised RNA tails which are removable by ribonuclease treatment. RNA size was determined before (3,000 nucleotides) and after (1,100 nucleotides) hybridisation suggesting that during hybridisation and subsequent isolation of hybrids the RNA is cut on average once or twice per molecule. Assuming the cuts are random and that there is no more than one repetitive element per bound fragment, then about 70% of the hnRNA molecules entering the reaction should contain one such sequence. If on the other hand some bound fragments were composed wholly of repetitive sequences and others contained smaller than average sized repetitive sequences this value will be lower but was calculated to be not less than 50%. Other interpretations, such as 75% of molecules composed of non-repetitive sequences and 25% composed of intermingled repetitive and non-repetitive elements, do not, however, seem to have been excluded.

Another point of interest is that the average size of repetitive and non-repetitive sequences in the hnRNA *Cot* 40 hybrids can be computed from the average length of hybridised molecules (1,100 nucleotides) and the proportion of hybrid which is resistant to ribonuclease (20–35%). Repetitive sequences in sea urchin gastrula hnRNA are therefore about 300 nucleotides long, a figure which is in good agreement with the average length of interspersed repetitive sequences found in sea urchin (Graham *et al.*, *Cell*, **1**, 127; 1974) and *Xenopus* DNA (Davidson *et al.*, *J. molec. Biol.*, **77**, 1; 1973).

Smith *et al.* next investigated the hybridisation of hnRNA to non-repetitive DNA sequences by allowing the hybridisation reaction to proceed beyond *Cot* 40 and up to *Cot* 4,000 at which point about 47% of the ^3H -labelled hnRNA is in hybrid; this value can be increased to 65–70% at very high DNA to RNA ratios. It is clear from the shape of the *Cot* curve that a large proportion of hnRNA molecules contain sequences transcribed from non-repetitive DNA. Although the detailed interpretation of these results suffers from some uncertainties—mainly to do with possible variation in specific activity of the reacting species—the suggestion is that a few unique sequences are present in hnRNA in much greater amounts than others, since only at very high DNA/RNA ratios are there enough sites provided for most of the RNA to hybridise. Most unique sequences in hnRNA are present in relatively few copies since there is essentially no increase in hybridisation at *Cot* 4,000 from a DNA:RNA ratio of 30 up to 15,000. This means that enough single copy DNA sequence is present at the lower ratio to hybridise the bulk of the sequence complexity in hnRNA. Although it is not possible directly to calculate non-repetitive sequence

complexity from these results (RNA-driven reactions are required for this), a rough minimum estimate of 10% of the unique sequence of the total genome is obtained. Preliminary results using RNA-driven hybridisation reactions indicate a value two or three times greater.

In order to compare the genetic information in hnRNA with that in polysome-bound mRNA it is necessary to estimate the proportion of unique DNA sequence complexity present in cytoplasmic mRNA. Galau *et al.* have done this for sea urchin gastrulae using RNA-driven hybridisation reactions with ^3H -labelled purified non-repetitive DNA as the probe. First they isolated polysome-bound RNA by procedures previously shown to extract most of the cytoplasmic mRNA essentially free of hnRNA although containing substantial amounts of ribosomal RNA (Goldberg, Galau, Britten and Davidson, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3516; 1973). This was hybridised with ^3H -labelled non-repetitive DNA in 3–40 fold excess RNA for various lengths of time to give RNA *C*₀t_s up to 60,000. Since DNA renaturation is not prevented subsequent analysis distinguished between DNA/DNA and DNA/RNA duplex. It was found with four different RNA preparations that a maximum of 1% of the total ^3H -DNA enters DNA/RNA hybrids. Proof that termination of the hybridisation reaction is due to exhaustion of DNA sequences complementary to mRNA was provided by reisolating unreacted ^3H -DNA and rehybridising it to a further sample of RNA. Only 0.02% formed new hybrid although when annealed with excess cold DNA most of it would still enter DNA/DNA duplex. The non-repetitive DNA probe contained as contamination a small amount of repetitive sequence and proof that the DNA in the RNA/DNA hybrid is non-repetitive DNA was given by reisolating the DNA from the hybrids and reacting it with an excess of sheared total DNA, when it renatured with the kinetics expected of unique sequence DNA. From these data in a calculation which allows for asymmetric transcription and the measured proportion of DNA unavailable for hybridisation, Galau, Britten and Davidson conclude that 2.7% of the total single copy DNA sequence is represented by RNA transcripts in gastrula polysomes. Assuming a number-average length for mRNA of 1,200 nucleotides, this means that about 14,000 different mRNAs are present at this stage of development. It was calculated that only about 8% of the mRNA (called the complex class) drives the hybridisation reaction and contains 90–95% of the total sequence complexity. The rest (called the prevalent class) contains only 5–10% of the sequence complexity since no early low RNA *C*₀t transition was seen during the course of the reaction. From these figures, together with estimates for the total amount of mRNA in gastrula polysomes, the average number of complex class mRNA molecules per gene at any one time was calculated at 340; for the prevalent class of mRNA this figure must be two orders of magnitude greater.

It is clear from the results of these two papers that the proportion of the unique DNA transcribed into hnRNA is at least four times and maybe ten times greater than the proportion in active polysomes of sea urchin gastrulae. There is considerable debate at present over the possible role of hnRNA as a nuclear precursor to mRNA. If it is, these results suggest that 75–90% of diverse unique sequence transcripts present in nuclear RNA do not reach the cytoplasm, that is processing restricts qualitatively with respect to sequence the mRNA appearing in the cytoplasm.

This result is particularly interesting as it provides evidence for control of gene expression at a level beyond transcription. But before this interpretation can be accepted it is important to ascertain what proportion of the unique DNA sequences code for protein, and also that the unique sequence transcripts present in hnRNA but absent from the cytoplasm are representative of sequences which do code for proteins and do not have some other function.

PETER J. FORD

Einstein in crisis?

IN spite of the great aesthetic and philosophical appeal of Einstein's general theory of relativity, it is still, after 50 years of widespread acceptance, one of the least well-founded theories in physics as far as experimental confirmation is concerned. Of the three classic tests of the theory, the most impressive concerns the motion of the planet Mercury. In the nineteenth century it was known to Leverrier that the perihelion of Mercury's orbit precessed around the Sun at a calculated rate of 43 arc seconds a century. Such a deviation from a perfect elliptical orbit, although very small, was none the less a significant enough departure from Newton's theory of gravitation to lead to the search for an undiscovered planet (which even had a name—Vulcan) which supposedly lay within the orbit of Mercury, and was responsible for perturbing its motion.

Vulcan was not found, and in 1915 Einstein published his theory of relativity which beautifully accounted for the discrepancy in Mercury's motion. This success must be regarded as the backbone of observational support for the general theory of relativity, so that any questioning of the result must be regarded with the gravest concern. To question the result is just what Dicke of Princeton University has been doing for some years, by directing his attention to the shape of the Sun. As a result of a long series of observations, Dicke claims to have detected a solar oblateness (a difference between the polar and equatorial radii) of $(4.51 \pm 0.34) \times 10^{-3}\%$. The details of the observations, together with a tentative theory of the solar interior, are published in a recent issue of *Science* (**184**, 419–429; 1974).

Dicke interprets the solar oblateness in terms of an uneven distribution of mass in the Sun's interior, leading to a small but significant quadrupole moment of $(2.47 \pm 0.23) \times 10^{-5}$. The presence of such a quadrupole moment would have a profound effect on the motion of Mercury; it would in fact reduce the perihelion precession by three arc seconds a century—a discrepancy of 7% from the relativity result.

Under these circumstances there are always a number of alternative theories of gravity waiting in the wings, and it is no coincidence that one of the more serious contenders also comes from Dicke. Known as a scalar-tensor theory, it is based on the idea that as well as the usual gravitational field which surrounds a massive object, there is an additional (scalar) field which adds a fraction, for example, *s*, to a body's weight. The value of *s* is not determined by the theory, but is generally held to be about 15–20%. Calculations using the scalar-tensor theory show that Mercury's perihelion would still precess, but at a somewhat reduced rate. If *s* is about 15%, the reduction is around four seconds of arc a century. Not surprisingly, Dicke believes that the scalar-tensor theory is in better agreement with the observations than is Einstein's theory, provided that the solar oblateness interpretation is accepted as well.

It is unlikely that a large oblateness would be believed by astronomers unless there were a fairly good theory of stellar structure to go with it, and Dicke outlines such a theory in his *Science* article. One likely explanation for a quadrupole moment is the possibility that the interior of the Sun might be rotating faster than the surface. To obtain an effect of the right magnitude, it is necessary to have a solar core rotating at 20 times the surface value.

It is thus necessary to account for the relatively slow rotation of the surface layers. Interestingly, there is a ready made explanation available, provided by the 'solar wind'. This consists of streams of high velocity charged particles continually shooting out from the surface of the Sun. Such particles would couple strongly to the Sun itself through any magnetic field present, and would have the effect of 'braking' the relatively tenuous outer surface of the Sun as the field twists up because of rotation.

Needless to say, the presence in the solar interior of a high differential rate of rotation raises deep problems concerning stability. Nevertheless, observations of the rotation rates of other stars slightly more massive than the Sun indicate that young stars do indeed rotate about 20 times faster than the surface of the Sun, but that this rate falls off progressively as the star ages, presumably as a result of magnetic braking. The crucial question though, is whether the interiors of the older stars (such as the Sun) are still spinning at a very high rate. Extraordinary evidence that this might be so is provided from an unlikely source.

In order that the solar surface continues to rotate under the breaking action of the solar wind, there must be a coupling between the rapidly spinning core and the surface, to provide a mechanism for the transfer of angular momentum. One such mechanism, involving turbulent diffusion of the solar fluid, carries angular momentum upwards, but also carries lithium downwards. Lithium is produced in certain quantities in all stars, but in the Sun there is apparently a depletion by a factor of 200 from the expected abundance near the surface. Dicke advances this as evidence of a rapidly rotating core.

These provocative ideas of Dicke have caused a great deal of controversy in recent years. Nevertheless, at this time, he feels that criticisms have been well met, and that Einstein's theory faces a very real crisis. Naturally, the case cannot be decided on the basis of solar oblateness arguments alone. The complexities of stellar structure, and our ignorance concerning the condition of the Sun below its visible surface layers, preclude such sweeping conclusions. One other possibility of discriminating between the scalar-tensor theory and general relativity concerns the value of the Newtonian gravitational constant G , which in the former theory is actually a time-dependent quantity. The extremely slow variation of G predicted by Dicke may soon be accessible to observation using radar-ranging techniques to measure the motions of the nearby planets. The importance of the issue at stake should lead to observations like these being vigorously pursued in the next few years.

P. C. W. DAVIES

Does the geomagnetic field affect climate?

ALTHOUGH the suggestion that there may be a relationship between climatic changes and variations in the Earth's magnetic field was first made more than 20 years ago, convincing correlations between the two have been discovered only much more recently. The surprise is not that it took so long but that it ever happened at all. For what possible connection could there be between phenomena so obviously disparate? Yet the correlations exist; and although a direct causal relationship may be genuinely doubted, the fact that climate and geomagnetic field are linked at all raises the need to identify the intermediate factors by which the correlations come about.

Apparently for historical reasons, some workers have drawn significance from the fact that both climate and magnetism seem to be related to fluctuations in atmospheric radiocarbon activity. De Vries (*Proc. K. ned. Akad. Wet.*, **B61**, 94; 1958) seems to have been the first to propose a correlation between radiocarbon activity and climatic changes, in which he was followed ten years later by Damon (*Met. Monogr.*, **8**, 151; 1968); but by Damon's time Elsasser *et al.* (*Nature*, **178**, 1226; 1956) had already pointed out that radiocarbon activity was likely to be influenced by changes in the magnetic field. The argument here is that the cosmic radiation entering the atmosphere to generate the radiocarbon will be reduced if the strength of the geomagnetic

field is increased, for the field has a shielding effect—and this phenomenon has received support from many subsequent workers. So variations in the intensity of the Earth's magnetic field fluctuations in atmospheric radiocarbon activity and climatic changes are apparently correlated for the past 7,000 yr, and there is a clearly understood relationship between the magnetic field and radiocarbon activity. Unfortunately, however, all this does nothing to explain just how and why climate comes to be involved.

More recently, important, albeit phenomenological, light has been thrown on the climate connection by the work of Wollin and his colleagues at the Lamont-Doherty Geological Observatory. Wollin *et al.* (*Earth planet. Sci. Lett.*, **12**, 175; 1971), for example, examined deep sea sedimentary cores for the past 470,000 yr and found that changes in the intensity of magnetisation and the palaeomagnetic inclination of the sediments could be correlated with the climatic changes in the same sediments as represented by variations in, for example, the abundance of *Globorotalia menardii* and the coiling direction of *G. truncatulinoides*. As a result, they tentatively concluded that a "cause and effect relationship links changes of the Earth's magnetic field and climate" (for example, that higher magnetic intensity produces, or is produced by, colder climate). They also suggested that "it is possible that magnetism may modulate climate to some degree by the shielding effect against solar corpuscular radiation provided by the Earth's magnetic field"—thus invoking a clear, if perhaps forced, analogy with the phenomenon known to affect cosmic rays. Later, Wollin *et al.* (*Nature*, **232**, 549; 1971) used other deep sea cores to extend the correlation between magnetisation variations and climatic changes back to 1.2 Myr.

The best way of summarising the work of Wollin and his colleagues on deep sea sediments would probably be to say that the correlations themselves are convincing whereas the explanation is not, which is why Amerigian (*Earth planet. Sci. Lett.*, **21**, 321; 1974) has sought to produce something "simple and less astonishing". Amerigian's basic point is that at least equally feasible causes of the observed correlations may be invoked, based on seafloor dynamic processes already known and known to be climatically variable. For example, one of the principal factors affecting the intensity of magnetisation of sediments is the intensity of magnetisation of the particles which settle to form the sediments in the first place, and this in turn depends on composition. Most of the cores examined by Wollin *et al.* were high in organic calcium carbonate and, as Amerigian shows in a scatter plot, the greater the proportion of essentially non-magnetic calcium carbonate present in a sediment the lower (statistically) is the sediment's intensity of magnetisation. In other words, calcium carbonate production, which is climatically controlled, can affect intensity of magnetisation in a way which is completely unrelated to the strength of the geomagnetic field. Under such circumstances it would clearly be unwise to suppose that a correlation between intensity of magnetisation and (other) climatic indicators necessarily implies one between geomagnetic field intensity and climate.

The second important factor influencing the intensity of magnetisation of sediments is the efficiency of the process by which the settling particles are aligned into the prevailing magnetic field direction, and this in turn is related not only to the strength of the field in which the deposition takes place but also to the grain size distribution of the magnetic material involved. Materials with different grain size distributions may settle out into the ambient field direction with different efficiencies (that is, the proportion of material attaining a magnetically controlled orientation will vary) depending on the current velocity. Thus if the bottom current velocity is related to climate, the intensity of magnetisation of the sediment will be partly related to climate. Again, then, it does not necessarily follow that a

correlation between intensity of magnetisation and climatic indicators means that the latter correlate with geomagnetic field strength.

Amerigian then goes on to show that the reported correlations between palaeomagnetic inclination and climatic indicators can result from variable inclination errors introduced by bottom-current velocity changes which are also climatically controlled. He does recognise, however, that his various mechanisms are only valid if the magnetisations of sediments are depositional rather than post-depositional, for climatically controlled depositional processes can obviously only be relevant to the observed correlations as long as sediment magnetisations arise at the time the sediment is formed. There are known to be several ways in which post-depositional magnetisations can be, and probably are, produced, although it is by no means clear that such processes are universal. In any case, Amerigian's aim seems to be less to push particular explanations or combinations of explanations, for the observed correlations than simply to demonstrate that the correlations need not be direct.

Where Amerigian may be on less certain ground is in his treatment of correlations between directly observed geomagnetic field changes and climatic variations. Since their work on sedimentary cores, for example, Wollin *et al.* (*Nature*, **242**, 34; 1973) have reported just such correlations, but Amerigian dismisses them because of what he takes to be "unsatisfactory" data selection. Whether or not he is justified in this particular instance, the fact is that other workers are also finding relationships between magnetism and climate. It is by no means clear that these will be so easily refuted.

PETER J. SMITH

Lymphocytes at war

It is a precept of many immunologists that the primary role of the vertebrate immune response is defensive, enabling the responding organism to resist attack by such foreign agencies as bacteria and viruses. Although there are objections to this notion—for example that invertebrates do not have such specific immune responses and yet they seem to defend themselves well—attack and defence have become so firmly rooted in the thinking of immunologists that many of their present-day analytical methods could be described as war games. One of the games with the most immediate impact is that which is fought *in vitro* between two genetically and thus antigenically distinct cell populations. It is supposed that the outcome of this battle in some way reflects the manner in which foreign mammalian cells are dealt with in the living animal after tissue or organ transplantation. There is also the idea that tumours are antigenically distinctive and thus the various kinds of stimulation which can be shown *in vitro* are a manifestation of an anti-tumour surveillance mechanism. Like many war games the moves of those played by immunologists are complex and irritatingly abstruse to the uninitiated. Three recent communications in *Nature* illustrate the genre.

Lafferty and his colleagues Misko and Cooley (*Nature*, **249**, 275–276; 1974) lay down the basic rule of their game in their first sentence. In translation it says that the response to a particular set of transplantation antigens *in vitro* is sometimes enhanced if there is a second and different set of antigens available to respond: two adversaries are better than one. Lafferty *et al.* cultured the cells from the lymph nodes of C57BL/6J mice with γ -irradiated BALB/c spleen cells. This is said to be a one-way mixed lymphocyte reaction in which the outcome is simplified by inactivating the capacity of one of cell populations to respond but leaving its stimulatory property apparently intact. The severity of the reaction can be measured either by

incorporation of tritiated thymidine into the cultures at an appropriate time or by subculture of the responder cells onto a ^{51}Cr -labelled tumour cell population which has some of the same surface antigens as the BALB/c stimulator cells (H-2^d).

Lafferty *et al.* go on to show that although γ -irradiated cells are adequate stimulators, ultraviolet-irradiated cells are not. It seems that ultraviolet-irradiated cells still have the relevant antigens as they are effective absorbers of the appropriate anti-H-2 antisera. If, however, to cultures containing responder C57BL/6J lymph node cells (H-2) and (poor) stimulator ultraviolet-irradiated BALB/c spleen cells (H-2^b) is added a γ -irradiated CBA spleen cell population (H-2^k), then the C57BL cells respond vigorously and generate anti-H-2^d cytotoxic cells. Lafferty and his colleagues think that their experiments raise the possibility that "the antigenic differences between donor and host may not constitute a major barrier to all transplantation" but that if the graft contains more effectively antigenic donor lymphocytes then a more vigorous response against the antigens of the major portion of the graft will ensue.

Dennert's experiments (*Nature*, **249**, 358–360; 1974) are like those of Lafferty *et al.* in that the DBA/2J mastocytoma (H-2^d) is again a target cell for attacking lymphocytes. Dennert injected the tumour into C57BL/6J mice and then assayed for cytotoxic cells in their spleens 9 days later. He found that if he used fresh tumour cells to immunise, then cytotoxicity developed but if he used cells fixed with glutaraldehyde or formaldehyde little cytotoxicity was discovered. Dennert then went on to estimate the immunological capacity of the spleens of the variously immunised C57BL mice for their 'helper' capacity by incubating them *in vitro* with trinitrophenyl (TNP)-coupled tumour cells and subsequently measuring the anti-TNP response by a plaque assay. If the spleen cells are immune anti the tumour cells then, because the tumour cells are acting as the carrier for the TNP hapten in the culture, an anti-hapten response should follow. Paradoxically the spleen from mice immunised with fresh tumour cells, which had in the other tests shown such a vigorous cytotoxic response, had little helper activity whereas those from mice immunised with fixed cells had helper activity but little cytotoxicity.

Dennert goes on to show that spleen cells with high cytotoxic capacity are also able to suppress the expression of helper function. These experiments serve to exemplify the intricacy of contemporary cellular immunology and Dennert feels that they suggest strongly that cytotoxic effector cells and helper cells are different 'subsets' of T (thymus-derived) cells in contradiction to the earlier suggestion of Kreth and Williamson (*Nature*, **234**, 454; 1971) that they were the same cells. Categorisation of heterogeneity of T cells is presently popular but it will remain to be seen whether the various putative classes are related to some basic diversity or represent alternative physiological conditions of initially the same cell populations. Thus Dennert's article raises the larger question of whether lymphocytes are heterogeneous before antigenic stimulation or even as a result of it.

The third of the trilogy by Plata and Levy (*Nature*, **249**, 271–274; 1974) draws attention to the fact that although all the effector cells in chromium release assays of cell-mediated cytotoxicity against murine sarcoma virus, induced tumours in C57BL/6 mice seem to be T cells, both T and non-T cells can be detected in the microcytotoxicity assay (Takasugi and Klein, *Transplantation*, **9**, 219; 1970). Furthermore, it proved possible to show that the capacity of T cells to kill as detected by chromium release was not blocked by serum from tumour-bearing animals whereas in the microcytotoxicity assay blocking occurred. Like Dennert, Plata and Levy wish to conclude that they are dealing with two populations of T cells distinguished by their functional capacities *in vitro*.

A. J. S. DAVIES

Pioneering Jupiter meeting at RAS

by John Gribbin

ON Friday May 24 the Royal Astronomical Society broke new ground with their first meeting devoted entirely to discussion of the planet Jupiter. The stimulus to hold such a meeting at present came, of course, from the historic data gathered by Pioneer 10 a few months ago, and which are now being analysed. The process of analysis and interpretation is a long one, and although some of the speakers at the meeting are, it seems, regular acquaintances from similar meetings held at various institutions every few weeks, they have yet to hear each other covering entirely familiar ground. So what emerged from the recent RAS meeting was not in any sense a definitive statement about the nature of Jupiter—rather, a progress report of the state of the art, which raises one or two extremely puzzling questions.

The meeting began with a resume from K. Runcorn (University of Newcastle upon Tyne) of the pre-Pioneer picture of Jupiter and its radioactivity. It is now some 52 years since Jeffreys produced the first model of Jupiter, Runcorn recalled, and 37 years since advances in quantum mechanics showed that metallic hydrogen can be formed at pressures comparable to those found in the interior of that giant planet. For some two decades the presence of radio emission from Jupiter has been known—indeed it is the brightest object in the radio sky—and this ties in well with the idea of a fluid metallic core in which dynamo processes operate.

No discussion of Jupiter is complete without mention of the Great Red Spot, which rotates with a period close to 9 h 55 min 40 s, some 10 s more than the rotation period of radio features. This can be explained, broadly speaking, by Hide's model which Runcorn called "the most physically satisfying explanation of the Great Red Spot". In this model, the spot is seen as the visible part of a Taylor column produced by an irregularity (mountain or crater) in the underlying solid surface. Then, the spot period represents the rotation period of the solid mantle, and the radio period the period of the weakly coupled core.

If that is the case, it is to be expected that slight variations in the two periods—a few milliseconds or so—could be correlated in much the same way that variations in the rotation of the Earth's magnetosphere can be correlated with changes in the length of day. Tantalisingly, the available radio observations can neither confirm nor deny this hypothesis, although to my eye the curves presented by Runcorn seem to fit this

proposal better than the alternative hypothesis, that the radio period is strictly constant. One thing the Earth-bound radio observations do imply is that the Jovian magnetic field is, if dipolar, inclined at about 11° to the rotation axis—and this provides a neat tie in with the Pioneer 10 data.

E. J. Smith (Jet Propulsion Laboratory, CalTech) described the Pioneer observations of the Jovian magnetic field. The data are not sufficient to define a unique model, but the simplest model which fits them is of a dipolar field, inclined at 11° and offset from the planet's centre, with a strength of 4 gauss at the equator. Closest approach of the vehicle to Jupiter was 2.8 Jovian radii, and the field showed a steady cube law dependence on distance for most of the trajectory, as expected for a dipolar field. The offset of the dipole from the centre of Jupiter seems to be about one tenth of the planet's radius (R_J), much more than the equivalent offset of the Earth's field.

The magnetosphere beyond some $20 R_J$ is strongly deformed from the dipolar form which is characteristically closer to the planet, and seems to contain a thin current sheet. This has an important effect on the distribution of energetic particles around Jupiter, as J. Simpson (Enrico Fermi Laboratory, University of Chicago) and R. W. Fillius (University of California, San Diego) reported.

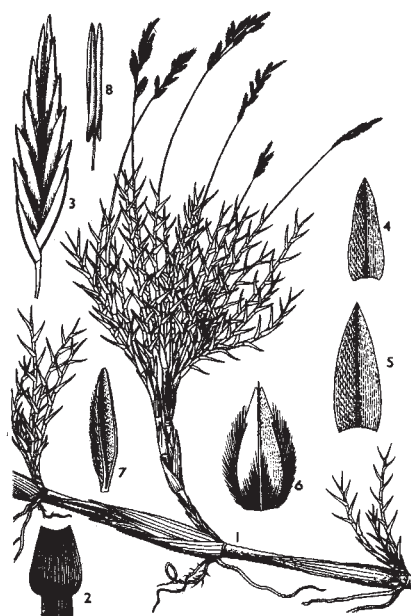
Much of the data concerning the particle distributions and the sweeping up effect of some of the Jovian satellites is already familiar from published work, and the latest developments add little to the earlier analyses. But two intriguing features remain to be explained. First, the distribution of both protons and non-relativistic electrons peaks slightly further out from Jupiter than the Pioneer 10 periapsis. Extrapolation of the measurements suggests that there is a 'particle hole' close to the planet, and this can hardly be attributed to the influence of Amalthea since high energy (35 MeV) electrons are not affected. Pioneer 11 is now targeted to fly through this hole, so its reality should soon be confirmed. If the distribution of protons does, however, peak again close in to Jupiter then Pioneer 11 is unlikely to survive.

But the most intriguing discovery mentioned at the RAS meeting concerns the bursts of relativistic electrons apparently ejected by Jupiter. These bursts were first detected by Pioneer 10 half a year before encounter, and got bigger as the spacecraft approached the planet. Clearly they are not solar particles, since they come from the wrong direction; the idea that they might represent interstellar particles leaking in to the Solar System from outside held some appeal at one time,

but now it seems clear that they do originate from Jupiter.

The crucial evidence is that once Pioneer 10 had made its dive through the Jovian magnetosphere it was possible to extrapolate back from observations of the roughly 10 h periodic variations produced by the rotation of the planet. This extrapolation showed that all the bursts seen on the way to Jupiter showed structure on a timescale of 10 h exactly in phase with the variations inside the Jovian magnetosphere. Clearly, unless Pioneer 10 had travelled in a trajectory specially favoured for particle ejection (which seems most unlikely), Jupiter must be radiating waves of particles outwards over a wide range of latitudes. This is very disturbing for established theories which suggest that particles should be nicely contained in the equatorial plane, and shows how much remains to be discovered about Jupiter. At the same time, this source of non-solar particles provides an invaluable new tool for investigating propagation of particles in interplanetary space. With Pioneer 11 still to make its (perhaps suicidal) close dive past Jupiter, it looks as if this will not be the last RAS meeting devoted exclusively to the giant of the Solar System.

East African grasses



The second part of the Graminae section of the *Flora of Tropical East Africa* (edit. by Clayton, Phillips and Renvoize; Crown Agents of Oversea Governments and Administrations, London, April 1974, £2.75) contains this drawing of *Odysea paucinervis* which is found south of the Congo River on saline soils between 1,800 and 1,900 m.

Ambiguous gravity

from Peter J. Smith
Geomagnetism Correspondent

THE chief difficulty in gravity interpretation is that gravity anomalies by themselves can never be used to determine subsurface structure uniquely. There is always a very large, and in theory infinite, number of structural solutions consistent with any observed anomaly, although in practice the quantity of possible structures may often be reduced to more manageable proportions by a combination of common sense, experience of comparable environments, and complementary geological, geophysical or geochemical data. In the end, however, the decision about the 'most reasonable' structure model is likely to depend as much on the quality and amount of the support data as on the gravity anomaly itself.

Disagreements over the interpretation of gravity are therefore common, especially where associated non-gravity data are poor; and this seems to be the situation in the Galapagos Islands. Last year, Case *et al.* (*Science*, **181**, 1040; 1973) reported that over the islands there is a residual negative anomaly of at least 80 mgal superimposed on a broader positive anomaly of unknown amplitude and extent; and they interpreted this in the light of geological evidence in terms of a low density subsurface region related to a hot spot or mantle plume.

But those earth scientists (and there are many) who do not believe in plumes may breathe again, for Watts and Cochran (*Science*, **184**, 808; 1974) have now come up with a model involving simply downwarping of the lithosphere under the weight of the islands—an alternative actually considered and not entirely discounted by Case and his colleagues themselves. Watts and Cochran do not claim their structure is necessarily the right one, nor that a plume does not necessarily underlie the Galapagos; their main point is to re-emphasise the inherent ambiguity of gravity data. Everybody agrees that the matter will not be resolved until other, preferably seismic, data become available.

Hedgerow removal and bird life

from Peter D. Moore
Plant Ecology Correspondent

IN 1962 it was estimated that there were 616,000 miles of hedges in Great Britain (Locke, *Q. Jl For.*, **56**, 137; 1962). This was calculated by Moore, Hooper and Davis (*J. appl. Ecol.*, **4**, 201; 1967) to represent an area of

448,000 acres, which was more than twice the area of the British National Nature Reserves of that time. It was further suggested in these reports that the number of breeding bird species in agricultural land was about twice as great in regions with hedges than in areas where hedges had been removed. Very naturally these authors regarded the extensive clearance of hedges in Britain as one of the principal detrimental events in land management in recent years and it had led in particular to a reduction in the quantity and diversity of bird life in rural districts.

These findings have been generally accepted, but Murton and Westwood (*Br. Birds*, **67**, 41; 1974) now question their validity. These authors claim that the overall diversity of birds in a 200-acre sample area of agricultural land in Cambridgeshire has changed little, and may even have increased, since it was last censused in 1960–61. This is despite a reduction in hedgerows to one third of their former extent and the reclamation of half of the area then considered 'rough and waste'. These results are bound to cause confusion among conservationists.

One should first ask what is meant by diversity in this context. Murton and Westwood use an index of diversity devised by Fisher, Corbett and Williams (*J. anim. Ecol.*, **12**, 42; 1943) which is based on the assumption that the frequency distribution of species ranked according to the number of individuals by which they are represented, follows a logarithmic series. Although this frequency relationship has often been demonstrated in plant and animal communities, such theoretical indices can be misleading; an empirically derived diversity measure would have been preferable (see Hurlbert, *Ecology*, **52**, 577; 1971).

Whatever the relative merits of diversity indices, one is still faced with the fact that during the ten year period, the total number of species recorded in summer rose from 63 to 66 (although 19 of these species had decreased in numerical abundance) and in winter fell from 65 to 59; in effect there was little change in the total number of species recorded. Murton and Westwood account for this by considering the hedgerow to be a suboptimal habitat for most species which breed in them. Hedgerow removal therefore results in either a retreat of the species to optimal sites (such as woodland) or a modification in the bird's breeding behaviour (blackbirds may nest among the stumps of the cleared hedgerow and along ditch banks). Less adaptable species such as the song thrush may decrease, but are often replaced by other species such as the reed bunting.

These explanations are very accept-

able but they do suggest that the area sampled is not typical of regions in which hedgerow clearance has been practised. It differs in the retention of woodland fragments, hedgerow stumps and ditches, all of which serve to maintain diversity. Their conclusions cannot, therefore, be construed as general principles relating to the widespread effects of hedgerow removal.

Lethal mutants in *Drosophila* development

from Benjamin Lewin
Molecular Genetics Correspondent

THE imaginal disks of *Drosophila* provide one of the most promising systems for the genetic analyses of development and the recent isolation of many lethal mutants in disk development is therefore an important step towards defining the components of the system. The disks are larval tissues which subsequently give rise to many of the adult tissues; they include wing, leg, antenna and eye disks. Each disk is thus determined to produce a particular adult structure, the differentiation of which is triggered on metamorphosis. The disks provide an interesting experimental system, for passage of disks through the abdomens of adult flies allows indefinite continued growth, without loss of the determined state (which can be tested by injected tissue into larvae and identifying the adult tissues formed from it after metamorphosis). Transdetermination occasionally takes place, when the state of determination of tissue changes from one disk type to another; and homoeotic mutants exist in which certain disks give rise to adult structures usually formed from other disks.

The independence of the programmes for larval and adult development in *Drosophila* is emphasised by the observation that mutants which prevent disk development may be lethal to the adult but need not affect the larval stage at all. The characterisation of lethal mutants which affect all the disks is reported by Shearn and Garen in the April issue of the *Proceedings of the National Academy of Sciences* (**71**, 1393–1397; 1974). The mutants fall into two classes: *diskless* mutants form no imaginal disks; whereas *small disk* mutants form some or all of the disks, but whichever are present are small, misshapen and cannot differentiate into adult structures.

Mutants were isolated by screening for lethals dying in late development after treatment with chemical mutagens. The 23 *diskless* and 34 *small disk* mutants fall into 52 complementation groups; 48 groups contained one

mutant, three contained two mutants each and one contained three *small disk* mutants. One of the groups with two mutants comprised two *diskless* phenotypes, one included two *small disk* types, and the third represented one of each phenotype.

Two types of developmental deficiency might influence all the disks: some feature of the larval environment essential for development of all disks might be lacking; or some autonomous function common to all disks might be defective. The first model can be tested by injecting disks of normal larvae into the *diskless* and *small disk* larvae to see whether normal growth is possible; the second model can be tested by injecting suspensions of cells from early embryos of the mutants into normal larval hosts to see whether adult structures derived from disks are present after metamorphosis.

Injection of cells from *diskless* early embryos into wild type hosts did not lead to development of any adult structures when the *diskless* mutant was homozygous; such structures can, of course, be formed by cells from *diskless*/+heterozygous embryos. Quite different results were obtained when cells were taken from *small disk* mutants—these could provide the complete range of adult structures specified by all disk types (whether the mutation was in homozygous or heterozygous state).

When this experiment was carried out in reverse, wild type disks injected into *diskless* mutant larvae appeared to grow normally; although this experiment cannot, of course, be taken to the desirable conclusion of following the differentiation of wild type disks in *diskless* larvae, when the injected disks were retrieved before larval death and then reimplanted into normal larvae, they developed normally during metamorphosis. But in contrast, wild type disks cannot grow in one *small disk* host, although they do develop normally on subsequent injection into a normal larva; and in another *small disk* mutant host, some growth (although apparently abnormal) takes place, and the disk then loses its ability to develop normally upon subsequent implantation in a normal host.

Small disk mutants therefore seem to possess competent imaginal disks at an early stage of embryogenesis, but the disks subsequently fail to develop normally because the environment is in some way defective. The developmental potential of the disks seems to be irretrievably lost by the third instar stage which immediately precedes metamorphosis. (But the phenotype of the *small disk* class seems to cover some heterogeneity; for example, the *small disk* and one *diskless* mutant behaves as a *diskless* host when wild type disks are

injected into its larvae.) The *diskless* mutants seem to represent a different type of defect; some function involved in the (autonomous) development of all disks must be mutant.

The selective system used to isolate these mutants applied only to the third chromosome; by applying a Poisson distribution to the number of complementation groups with one and two members, Shearn and Garen inferred that there may be almost 400 further (at present unidentified) groups on this chromosome. This would imply that the genome of *Drosophila in toto* may possess about 1,000 complementation groups implicated in disk development—about one fifth of the total estimated number of complementation groups. Although the sample of isolated mutants is not large enough to make this an accurate estimate, it clearly implies that a large proportion of the genes of *Drosophila* may be concerned with the development of imaginal disks, an important conclusion. Isolation and mapping of further mutants may therefore be profitable.

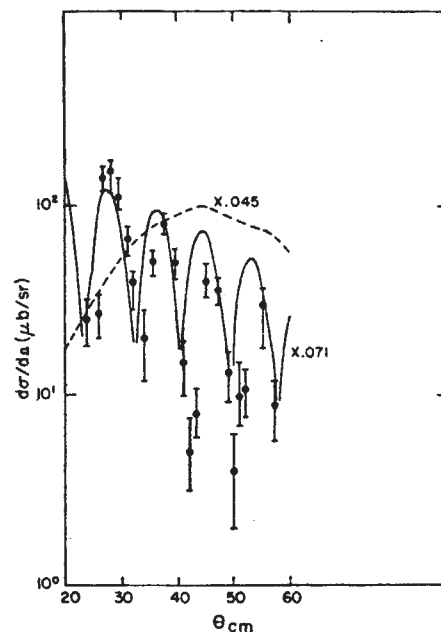
Most important, of course, will be experiments to implicate particular genes in defined pathways of disk development. And in addition to the many autonomous functions common to all disks, revealed by the *diskless* mutant class, there must be gene functions specific for each state of determination. Mutants defective in development of an individual disk type would therefore be useful, although difficult to obtain. And increasing success with genetic analysis must in turn emphasise the need for biochemical analyses of the disk system, a problem which at present looks difficult to overcome.

Transfer of excited alpha particles

from Peter E. Hodgson
Nuclear Theory Correspondent

MANY studies are now being made of the reactions in which an α particle is transferred from one nucleus to another, and these are providing important new information on nuclear structure. Some recent work by Charlton and Robson (*Phys. Rev. Lett.*, **32**, 946; 1974) has now provided evidence to suggest that the α particles can sometimes be transferred in an excited state and not only in its ground state as was previously assumed.

They analysed the differential cross section for the $^{36}\text{S} (^{16}\text{O}, ^{12}\text{C}) ^{36}\text{Ar}$ reaction obtained by Braun-Munzinger and colleagues in Heidelberg (*Phys. Rev. Lett.*, **31**, 1423; 1973). Some of these results are shown in the figure and it is notable that the cross section has a strongly oscillatory structure.



Differential cross section for the reaction $^{36}\text{S} (^{16}\text{O}, ^{12}\text{C}) ^{36}\text{Ar}$ at 30 MeV to the ground state of the residual nucleus compared with distorted wave calculations assuming the transferred α particle to be in its ground (---) and in its excited 2^+ state at 28.5 MeV (—). In ---, $V=100$; $W=30$, $r_0=1.22$; $r_1=1.25$; $a^*=0.5$; $a^1=0.4$. In —, $L_c=18$; $\Delta L=0.8$. The numbers on the curves are the spectroscopic factors obtained by normalising the calculated curves to the experimental data.

The cross sections for α -transfer reactions between nuclei can now be calculated quite well by the finite-range distorted-wave theory, provided the recoil effects are taken into account. Such calculations were made and gave a rather structureless distribution with a broad peak at about 45° as shown by the dashed line in the figure. This is in accord with semi-classical calculations, which give a bell-shaped curve centred at an angle corresponding to the scattering of a particle whose orbit just grazes the nuclear surface. This behaviour is completely different from that found experimentally.

Further calculations were then made taking into account the possibility that the α particle is transferred in an excited state. The 0^+ level at 20.2 MeV and the 2^+ level at 28.5 MeV were considered, as these satisfy the required parity and isospin selection rules. Estimates using harmonic oscillator wavefunctions indicated that reactions with the α particle in the 2^+ state are about a hundred times stronger than those with the 0^+ state, so only the former was considered.

The excited states of the α particle were assumed to be made up of pairs of like nucleons with the smallest possible internal motion, and the quantum numbers for the relative motions of the pairs give the required total spin.

In the dissociation $^{36}\text{Ar} \rightarrow ^{32}\text{S} + \alpha^*$, the four valence nucleons were assumed to be in the $0d_{3/2}$ orbit and the probability of the α particle being in an excited state was estimated using both the stretch scheme and the simple pairing model. Both calculations indicated that the α particle is more likely to be found in an excited state. The corresponding spectroscopic factors indicate that the transfer of an α particle in its 2^+ state is about five times more likely than the transfer in its ground state for the reaction considered.

The distorted-wave calculations for excited α -particle transfer were carried out in the standard way and used absorbing potentials depending on the orbital angular momentum in the way required by analyses of the elastic scattering of heavy ions by nuclei. The results are shown by the full line in the figure, and agree very well with the experimental data. Similar results are found for the corresponding reaction to the lowest 2^+ state of ^{36}Ar .

This is clearly a significant result that will stimulate many more investigations. In principle, the ground and all the excited states of the α particle should be added coherently to give the distorted-wave amplitude. More detailed analyses of similar reactions on other nuclei should provide further evidence of excited α transfer but the results of Charlton and Robson provide good evidence that such processes can take place.

Synchrotron radiation users meet

from G. V. Marr

THE electromagnetic radiation emitted from high energy electron synchrotrons, as a consequence of radially accelerating the electrons, is now well established as a means of studying the interaction of ultraviolet and X radiation with matter in its various forms. The first international symposium for synchrotron radiation users was held at the Daresbury Laboratory in January 1973 (see *Nature phys. Sci.*, **242**, 1; 1973) following the move of United Kingdom users from the Glasgow synchrotron to NINA in 1972. During the past year, demand for synchrotron radiation has increased and the conference held at the University of Reading from April 1 to 2, under the auspices of the spectroscopy group of the Institute of Physics, provided a means whereby the UK users could discuss the relevance of this source. The contributions covered present and future synchrotron radiation sources, applications to atomic, molecular, chemical

and solid state physics, biological studies, chemistry, X-ray optics and radiometry. The conference concluded with an open meeting with Science Research Council representatives to discuss the SRC paper "A Plan for Future Research with Synchrotron Radiation based on a Dedicated Storage Ring".

The main energy loss from the electron synchrotron is in the form of electromagnetic radiation whose spectrum extends continuously from the infrared to the X-ray region. It provides an intense source of high energy photons, predominantly polarised in the plane of the electron orbit and, because of the bunching of the electrons, pulsed at the accelerator radio frequencies. The radiation is emitted under good vacuum conditions and with divergences from the orbit plane comparable with a laser beam (see *Endeavour*, **33** (119) 55-66, May 1974).

Facts and figures for existing synchrotron sources were provided by I. H. Munro (Daresbury and University of Manchester) who outlined criteria for the ideal synchrotron source based on design work for the dedicated electron storage ring now under consideration by the Science Research Council. Progress with existing synchrotrons in atomic and molecular physics was introduced by K. Codling (University of Reading). In addition to energy level analysis, accurate absolute cross-sectional data are being provided on photoionisation processes and new experiments which allow photo-electron analysis to be carried out with respect to the E vector of the ionising radiation are now in progress. Continuous variation of the photon energy is providing data which will extend knowledge of configuration interaction, molecular orbitals and vibronic coupling; a point which was taken up by S. Leach (University of Paris, Orsay) in his review of applications in chemical physics where excited state relaxation processes in molecules and solids can benefit from lifetime studies using the modulated nature of the ultraviolet radiation of the synchrotron.

In the field of solid state physics, A. J. Forty (University of Warwick) reported experiments on absorption and reflectivity measurements which are contributing significantly to knowledge of band structures. Photoemission from surfaces is also providing new possibilities in metallurgy. D. A. Urtch (Queen Mary College, London) said that chemists on the other hand were exploring the possible use of synchrotron radiation to study the atomic components of molecular orbitals by way of X-ray emission spectroscopy. The new ring would allow an extension

of current work to the light elements where X-ray emission is less favoured. The polarised nature of the radiation offers a valuable new tool in studies of single crystal structures throughout the ultraviolet and X-ray regions. Of more immediate interest to molecular biologists, however, are the intensities of some 10^{-10} over conventional continuum X-ray sources that could become available. J. C. Hazelgrove (Medical Research Council, Cambridge) discussed advantages of these radiations in studies of transient states where it is important to have detailed short exposure X-ray diffraction pictures of specimens whose decay under normal sources alters vital characteristics during the time of exposure.

On the instrumental side, P. J. Key (National Physical Laboratory) said that the synchrotron, as a standard of relative spectral distribution throughout the near ultraviolet to the X-ray region, is becoming well established and radiometry problems are being studied. A discussion on X-ray optics introduced by M. Hart (University of Bristol) showed that fluxes some 10^5 greater than obtainable with conventional sources can be expected when the polarised nature of the synchrotron radiation is exploited. Quarter wave plates, crystal spectrometers and the development of X-ray diffraction gratings were also discussed.

In spite of the progress made and the enthusiasm of the users, it was evident that NINA and the other synchrotrons, where the users are parasitic to the experiments of the high energy nuclear physicists, are not ideal sources. The parasitic mode of operation is extremely inefficient where users are unable to control the quality and duration of the synchrotron beam. The SRC plan would seem to meet the foreseeable needs of the Reading conference and it was with this in mind that they attended the SRC meeting. Quite properly questions were asked relating to costs, location and long term usefulness of the proposed storage ring. The scientists present were particularly concerned that delays at this stage could result in the United Kingdom being without a source of synchrotron radiation if present plans concerning NINA are adhered to. A preliminary estimate of between 12 and 18 months for the level of funding in the SRC document was given at the meeting. It was agreed that there is every need to impress upon the SRC a sense of urgency in proceeding with the plan beyond the design stage, in order to ensure that the period between the close down of NINA as a synchrotron source and the start up of the new storage ring is kept to an absolute minimum.

Midwinter sunrise at Newgrange

J. Patrick

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At Newgrange on the morning of the winter solstice the Sun's rays shine through a specially contrived slit in the roof, along a 19 m passage to illuminate the central burial chamber. This passage grave was built about 1,000 yr earlier than the third phase of Stonehenge, by a different culture. It supports the claim that prehistoric cultures had studied the cycles of the Sun and utilised them in orientating their monuments.

NEWGRANGE is a passage grave in a Neolithic cemetery in County Meath, Ireland, about 30 miles NNW of Dublin (0 301 703). Two large samples of charcoal which were collected from caulking in between the roof slabs, yielded dates of 2475 ± 45 b.c. and 2465 ± 40 b.c. respectively. Depending upon which correction curve is used, this gives an age of 3100 ± 100 BC for the building of the tomb. It consists of a huge, artificially constructed cairn of water-rolled stones, which is approximately 80 m in diameter. A passage, 19 m long, runs into the mound

to give access to a large chamber about 3 m square and 6 m high. There are three recesses that open into the chamber, so that the ground plan has a cruciform appearance (Fig. 1). In each of these recesses is a large stone basin which was used to hold the burnt remains of the interred occupants.

Carvings

The tomb has been open to visitors since 1699 and an unknown amount of the original burial deposit may have been removed since then. In 1967 excavation of the tomb floor in front of the basin stones revealed the burnt bone fragments of about five people. These were accompanied by some grave goods characteristic of this type of tomb in Ireland, including stone pendants and beads, stone 'marbles' and bone points.

At the base of the mound is a continuous kerb of large slabs. It is believed that dry-stone walling was built on top of the kerb to a height of 3 m and that this wall was made of quartz for about 30 m on each side of the entrance. The sun on the

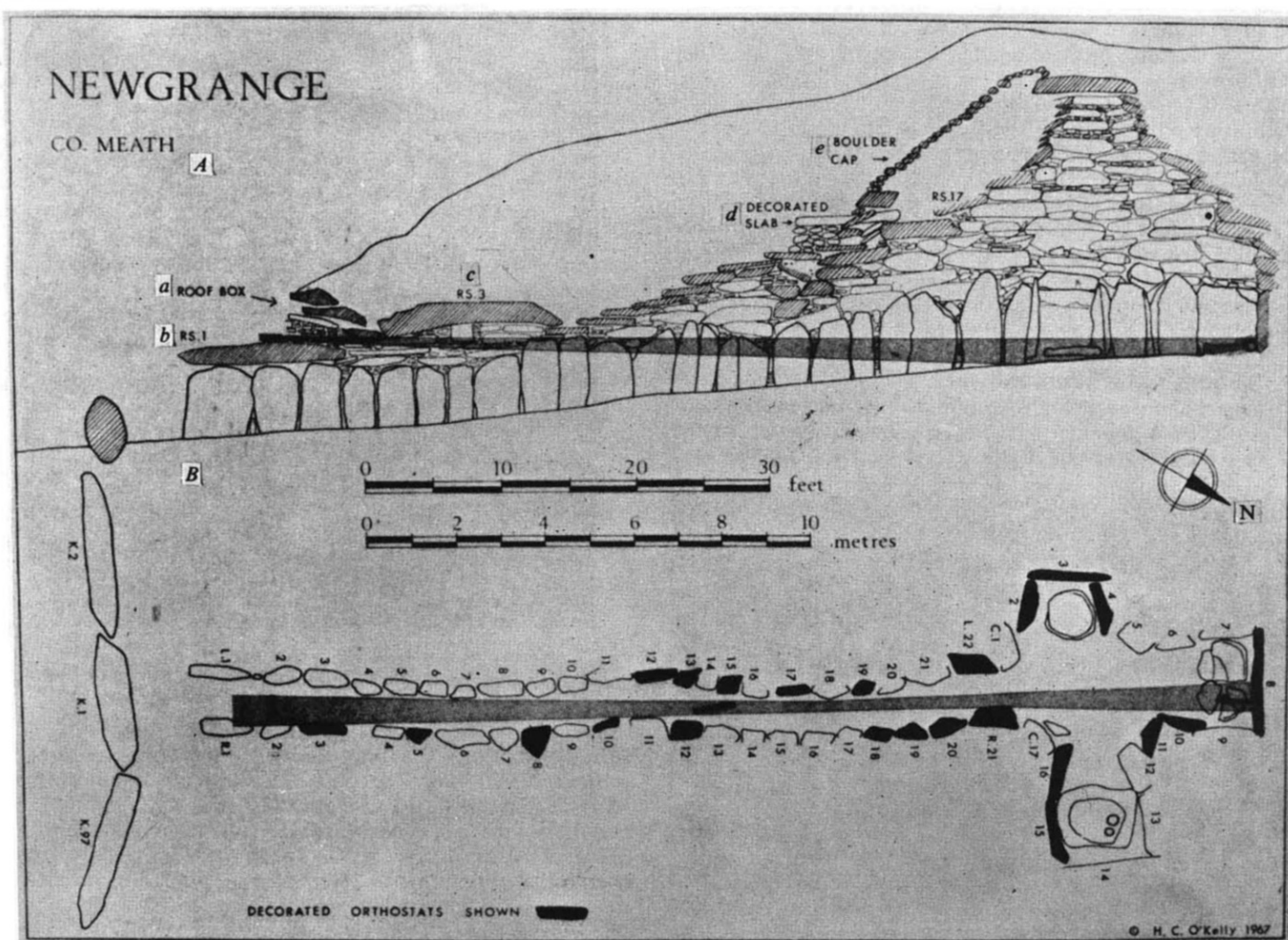


Fig. 1 The passage-grave at Newgrange, County Meath. *A*, Sectional elevation: *a*, roof box; *b*, roof slab 1; *c*, roof slab 3; *d*, decorated slabs; *e*, boulder cap. *B*, Plan view: wall orthostat stones are numbered; black shading, decorated orthostats; grey shading, range of path of Sun's rays during mid-winter solstice. (From ref. 1.)



Fig. 2 The decorated Entrance Stone in front of the burial chamber. The roof box can be seen, surmounted by the lozenge relief carving. (Green Studio.)

glistening white quartz would have presented a spectacular sight from the surrounding countryside. Most of this wall collapsed soon after completion of the monument, and the kerb stones became completely buried and remained hidden until uncovered again at the start of this century. Outside the kerb, there are 12 large standing stones that may form part of a circle, but their true relationship with the mound is not known for certain. They may be older than the passage-grave.

One of the most outstanding features of Newgrange is the decoration on the stones. The Entrance Stone, set in front of the passage, is regarded as one of the most impressive pieces of Megalithic art in Europe (Fig. 2). The artist who executed this piece of work has succeeded in using the shape of the stone to the utmost advantage in creating its aesthetic appeal. Another small but remarkable design is the three-spiral figure in the rear recess (Fig. 3). There are many other decorations within the passage and chamber, and on the kerbstones, virtually making Newgrange a Megalithic art gallery.

Astronomical alignment

Perhaps the most interesting feature of Newgrange is the 'roof box'. Dry stone walling has been constructed on top of the passage orthostats to support the second roofslab at a higher level



Fig. 3 The three-spiral figure in the rear recess of the chamber. (Green Studio.)

than the first, leaving a gap about 20–25 cm high and 1 m wide (Figs 1 and 2). The gap is protected from the weather by a box like structure of slabs supported on dry walls. This is open to the front. For further protection against the weather, the rear edge of the first roof slab and the front edge of the second roof slab have water channels cut on them. The leading edge of the uppermost roof slab of the roof box has an excellent relief carving of lozenges (Fig. 2).

During the winter solstice in 1969 Professor M. O'Kelly observed that 4 min after sunrise the Sun's rays shone through the roof box, along the passage, and up to the rear recess of the burial chamber, which became fully illuminated (Fig. 4). The spectacle lasted for 17 min before the sun moved out of alignment. Later, Mrs O'Kelly recalled a tradition that at a certain time of the year the sun lit up the three-spiral figure in the end of the chamber¹.

In 1972 Professor O'Kelly asked me to make an accurate survey of the roof box to see if this phenomenon would have occurred when the burial chamber was first built. The passage

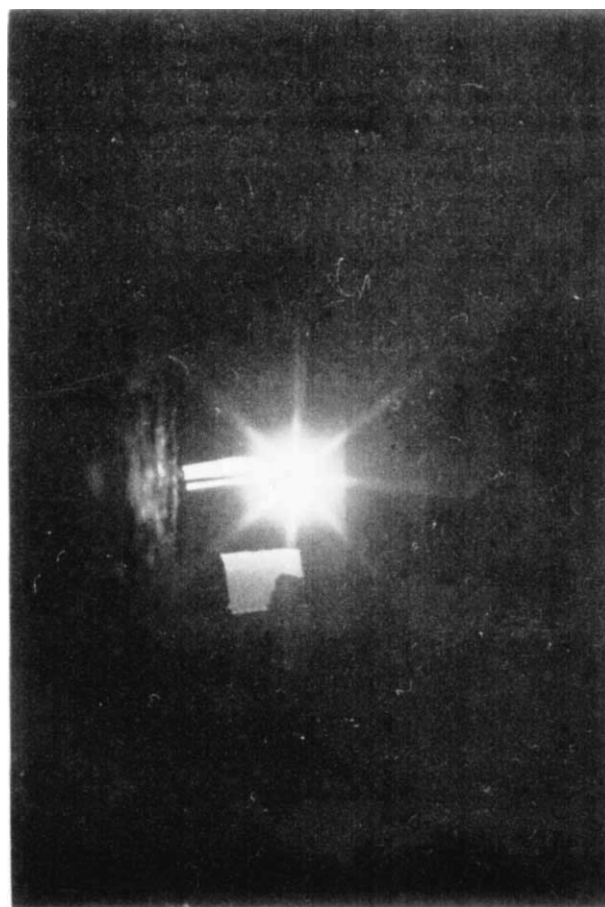


Fig. 4 Sunlight shining through the roof slit during mid-winter solstice. (M. J. O'Kelly.)

is in the form of two curves (Fig. 1), so that for a ray of light to travel directly from the roof box to the back wall of the rear recess it must be in the azimuth range $133^{\circ} 42' - 138^{\circ} 24'$. The elevation of the distant horizon ($0^{\circ} 51'$) is the minimum elevation of which the Sun's direct rays can enter the slit. The floor of the chamber is about 15 cm lower than the roof box, so at the minimum elevation sunlight will extend across the floor and into the rear recess. Light rays will not enter the chamber when the elevation exceeds about $1^{\circ} 40'$. This range of azimuths and elevations, reliable to about $15'$ and $5'$ respectively means that the Sun's rays will shine directly into the chamber if its declination lies between $-22^{\circ} 58'$ and $-25^{\circ} 53'$. It therefore seems that

the Sun has shone down the passage to the chamber ever since the date of its construction and will probably continue to do so for ever, regardless of secular changes in the obliquity of the ecliptic. It also means that the spectacle occurs for a number of days before and after the winter solstice.

Unfortunately, the vagaries of time have had their effect on the passage and some of the stones are now leaning inwards, thus trimming down the width of the beam of light. At the time of construction the beam would have been about 40 cm wide whereas now it is only 17 cm. The two principal orthostats causing the obstruction are L18 and L20 (Fig. 1). The first 10 orthostats on either side of the passage have been straightened but there is no way of straightening the rest without dismantling the whole structure.

There are about 200 passage graves in Ireland with many more allied tombs in Scotland and Brittany, but to my knowledge none of them has a roof box like that at Newgrange. As this structure is unique, and as the whole monument is so grandiose, it seems likely that its orientation is deliberate.

The monument best known for its astronomical orientations

is Stonehenge on the Salisbury Plain. After it had been built, it was remodelled several times, and the main astronomically orientated structures were erected in its third phase, which is dated at around 1800–2000 BC. This is about 1,000 years after the construction of Newgrange, and there is little connection between the two monuments or between the cultures which built them. The unambiguous definition of both direction and altitude at Newgrange is by far the most convincing evidence that some Megalithic structures were deliberately orientated on astronomical phenomena. This must lend greater credence to the theory that different cultures in the British Isles were investigating the basic solar cycles. It is quite possible that data passed between different cultures, so that Stonehenge eventually became an important repository of astronomical knowledge.

I would like to thank Professor and Mrs O'Kelly for their assistance and advice.

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¹ O'Kelly, C., *Illustrated guide to Newgrange*, second ed., 94–95 (John English, Wexford, 1971).

Structure and orientation of an RNA polymerase operon in *Escherichia coli*

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The genes for at least two Escherichia coli RNA polymerase subunits are contained in a single operon.

THE DNA-dependent RNA polymerase is a key enzyme in expression of the hereditary material. In *E. coli* it consists of at least four different subunits α , β , β' , and σ . The structural gene for the β subunit (*rif*) has been precisely located on the genetic map because mutations conferring resistance to the antibiotic rifampicin also result in the synthesis of RNA polymerase molecules altered in this polypeptide¹. Here we present evidence based on deletion studies, indicating that the structural gene for at least one other subunit, β' , is linked to the *rif* gene, and that both share a common promoter (P). These elements map clockwise on the bacterial chromosome in the order *P-rif-β'*. Thus our results provide a clear genetic basis for the suggestion made by Matzura, Molin and Maaløe that β' and β may be synthesised on a single polycistronic mRNA². In addition, they show that the structural genes and the promoter for this mRNA map clockwise on the bacterial chromosome, in the order *P-rif-β'*.

Because and deletion affecting RNA polymerase would be lethal in haploid cells³, we have constructed strains partially diploid for the *rif* region of the chromosome by introducing into the cell an F' factor which carries extensive amounts of chromosomal DNA on both sides of this locus (Fig. 1). Thus deletions into *rif* do not affect cell viability. Moreover we have chosen to isolate such deletions on the F' factor, because they can easily be characterised by transferring the episome into a variety of appropriately marked strains. To

obtain information about the structure and orientation of the operon, we have isolated a specific episomal deletion ending within the *rif* gene, and which extends leftwards into the nearby *argCBH* operon (Fig. 1). This deletion, which has removed all the DNA immediately to the left of *rif*, has nevertheless left the β' gene carried by the F' completely intact. This result is expected if the β' gene were adjacent to *rif* and located to its right. Moreover, this deletion also places the synthesis of the β' subunit under the control of the *argCBH* operon. Since *argCBH* is read in a clockwise fashion⁴, we conclude that the β' and β genes are both read in that direction.

Isolation of deletions into *rif*

We have developed a simple method for selecting deletion mutations on an F' factor which simultaneously affect the *rif* gene and its neighbour, *bfe* (ref. 5); mutations in these loci confer resistance to antibiotic rifampicin and the virulent bacteriophage BF23 respectively. Mutations to resistance in either gene are normally recessive to the wild-type allele (ref. 3 and L.E. and J.G.S. unpublished). Thus, a partial heterodiploid of the *bfe^r rif^r/F' bfe^s rif^s* is sensitive to both BF23 and rifampicin. Mutants with deletions inactivating the wild-type *bfe* and *rif* genes carried by the F' will, however, become resistant to both BF23 and the antibiotic since the only intact *bfe* and *rif* genes remaining in the cell are those conferring resistance. We can therefore readily select for such rare mutants by plating cultures of the partial heterodiploid on solid medium containing rifampicin,

Table 1 Bacterial strains

Name	Genotype	Source/reference
AS13	F ⁻ <i>metB arg</i> (C-B) ^{sup102} <i>purD his str^r spc^r gal mal lac</i>	A. Silverstone
AW1	F ⁻ <i>metB recA56</i>	S. Austin
HTA1	F ⁻ <i>his⁻ trpA9761</i> (amber) <i>argE171</i>	S. Baumberg
HTA19	F ⁻ <i>his⁻ trpA9761</i> (amber) <i>argB2</i>	S. Baumberg
LE104	<i>metB arg</i> (C-B) ^{sup102} <i>purD rif^r_{A1} bfe^r recA56</i> <i>str-r lac gal</i>	See below
LE109	<i>metB arg Δ</i> (C-B) ^{sup102} <i>purD rif^r_{A2} bfe^r recA56 str^r</i> <i>lac gal malA/KLF10 Δ18</i>	Text
LE113	<i>metB recA56/KLF10⁺</i>	LE104 × AW1
LE114	<i>metB recA56/KLF10 Δ18</i>	LE109 × AW1
LE115	<i>metB recA56 argR</i>	A spontaneous <i>can^r</i> derivative of AW1
LE116	<i>metB recA56 argR/KLF10⁺</i>	LE104 × LE115
LE117	<i>metB recA56 argR/KLF10 Δ18</i>	LE109 × LE115
LE210	F ⁺ <i>ara</i> (amber) <i>lac_{L125}</i> (amber) <i>galK_{U42}</i> (amber) <i>trp</i> (amber) <i>str^r metB SuIII IA2(P) rif^r_{ampD12} λ⁺</i>	Derived from MB204 (ref. 17)
LE213	F ⁻ <i>ara</i> (amber) <i>lac_{L125}</i> (amber) <i>galK_{U42}</i> (amber) <i>trp</i> (amber) <i>str^r metB SuIII A2(P)* rif^r_{ampIII/8} λ⁺</i>	Derived from MB204 (ref. 17)
MN8A	Hfr P4X <i>metB argB2 λ⁺</i>	S. Baumberg
P4X C53	Hfr P4X <i>argC53 λ⁺</i>	S. Baumberg

The genotypes of our bacterial strains are shown in Table 1. The strain LE104 was specially constructed for our deletion selection. It was made from AB2147 *ilv argH metB thi his lacY gal xyl malA tna str^r* (Wechsler, personal communication). In a series of steps the following markers were introduced into AB2147 (Silverstone and Errington, unpublished): *purD* (cross against a *purD* derivative of Hfr AB311 (ref. 18)); *Δarg*(C-B)_{sup102} (cross against Hfr P4X sup102 (ref. 6)); *rif-r* (spontaneous mutation); *recA56* (cross against Hfr KL16 *recA56* (ref. 19)); *bfe-r* (spontaneous mutation); *KLF10⁺* (cross against AW2).

* The pattern of *ara* and *lac* suppression in this strain is slightly different from that seen in LE210. However its suppressor is still temperature sensitive, since the strain does not grow on nutrient plates at 42°C.

spread with phage BF23 (Table 2).

The starting strain, LE104 (Table 1), carries the KLF10 episome which spans the *argCBH-metB-rif-purD* region of the chromosome (Fig. 1). The strain is Rec⁻ to prevent recombination between the episome and the chromosome and, to ensure that the cells retain the KLF10, the chromosome contains mutations in both *metB* and *purD*. We can also determine whether the *bfe-rif* deletions extend into the nearby *argCBH* operon, because the chromosome of this strain carries an Arg⁻ mutation (*sup102*)⁶; deletions terminating before *argCBH* leave the cells Arg⁺, whereas deletions extending into the *arg* operon will make the cells Arg⁻.

We have found that mutants having the desired Bfe^r Rif^r

phenotype arise spontaneously at a frequency of about 3×10^{-8} . The following observations show that these mutants are due to deletions on the F' factor (Table 2). First, many of them (63%) are Arg⁻ and have therefore lost, in addition to *bfe* and *rif*, one or more of the *arg* genes from the episome. Moreover, these Arg⁻ mutants do not revert to Arg⁺ (<10⁻⁸). Secondly, crosses in which the mutant F' factors are transferred to recipients carrying mutations in *argE*, C and B generate a deletion map (Table 2) congruent with the known map of the *arg* operon. Three of the deletions, Δ7, 12 and 18, enable us to order two amber *rif^r* mutations, *rif^r_{ampD12}* and *rif^r_{ampIII/8}*, which affect the β subunit of RNA polymerase (ref. 7 and R.S.H., S. J. Austin, and J. G. S., unpublished).

Table 2 Isolation and characterisation of *bfe-rif* deletions

Deletion on KLF10	Frequency*	Wild-type recombinants formed in crosses against recipients containing:							
		<i>metB</i>	<i>argE171</i>	<i>argC53</i>	<i>argB2</i>	<i>argH119</i>	<i>rif^r_{ampIII/8}</i>	<i>rif^r_{ampD12}</i>	<i>purD</i>
Δ4	1	+	—	—	—	—	<0.001	<0.01	+
Δ7	1	+	—	—	—	—	<0.001	0.03	+
Δ12, Δ18	2	+	+	+	—	—	<0.001	0.20	+
	11	+	+	+	+	nt	nt	nt	+
Partially characterised	12	+	—	nt	nt	—	nt	nt	+
	3	+	+	+	—	—	nt	nt	+
Wild type	+	+	+	+	+	+	100	100	+

Isolation of deletions affecting *rif* and *bfe*. Cultures (5 ml) of LE104 (Table 1), grown overnight in minimal salts²⁰ containing glucose (0.2%) thiamin (10 μg ml⁻¹) and arginine (20 μg ml⁻¹), were mixed with the virulent phage BF23 (multiplicity of infection = 10) and shaken at 37°C (3 h). They were then concentrated to 0.3 ml by centrifugation and spread in soft agar (2.5 ml) containing 10¹¹ p.f.u. of BF23 on three minimal agar plates containing arginine (20 μg ml⁻¹), thiamin (10 μg ml⁻¹) and glucose. After 4 h at 37°C each plate was overlaid with soft agar (3 ml) containing rifampicin (1.2 mg ml⁻¹), giving a final concentration in the plate of 100 μg ml⁻¹. The plates were incubated at 37°C for 2 d.

Characterisation of deletions. Each mutant carrying a deletion on KLF10 was mated against appropriately marked recipient strains by cross-streaking on selective medium⁷. Some combinations were not tried (nt).

Crosses against Arg⁻ recipients. The genotypes of the recipient strains, HTA1, P4XC53, MN8A and HTA19 are listed in Table 1. Deletion strains able to give Arg⁺ recombinants (+) give confluent growth in the cross streak. Those unable to give recombinants (—) show no growth at all. Note that both *argC53* and *argB2* are in Hfr strains which only permit the recovery of chromosomal recombinants²¹.

Crosses against *rif^r* mutants. Each donor was crossed against *rif^r* recipients (LE210, 213, Table 1) containing a temperature-sensitive amber suppressor SuIII (A2(P))²². Met⁺ derivatives containing each mutant KLF10 were recovered at the permissive temperature (30°C) on selective medium. They were then tested for the ability to generate *rif^r* recombinants able to grow at the restrictive temperature (42°C). In this table we show the percentage of ts⁺ cells in a colony of each derivative. Control colonies of the recipient *rif^r_{ampIII/8}* (LE213) and *rif^r_{ampD12}* (LE210) gave no more than 0.001% and 0.01% ts⁺ revertants respectively.

Crosses against a *metB purD* recipient. Our initial selection required that our deleted KLF10 factors retain *metB* and *purD* (see text). That they do retain these markers was confirmed in crosses against AS 13 *metB purD*.

* Number found in a total of 30 mutants recovered from a single culture of LE104.

† Deduced from the Arg⁺ phenotype of the parental deletion strains in this class.

Operon containing the *rif* and β' genes

One of the mutants selected from LE104 (Table 1), can give Arg⁺ recombinants on transfer of the F factors to *argC53*, but not *argB2*, recipients (Table 2). Consequently its deletion, $\Delta 18$, must have one end in the *argC-B* region of the episome. The other is between *rif*^{amD12} and *rif*^{amIII/8} (Table 2). If the β' gene lies just to the right of *rif*, $\Delta 18$ should inactivate *rif* but still leave the β' gene intact. Cells containing KLF10 $\Delta 18$ would thus be expected to have two copies of the gene for β' and only one for β (located on the chromosome).

We assay the β and β' subunits of RNA polymerase by electrophoresis of pulse-labelled, whole cell lysates on polyacrylamide gels containing sodium dodecyl sulphate, which separates the large β and β' subunits from other cell proteins (Fig. 2)². We have studied a number of KLF10 $\Delta 18$ diploids in this way. Their behaviour (see below) shows that KLF10 $\Delta 18$ still determines β' subunit synthesis, and implies that the β' gene lies just to the right of *rif*.

Evidence has previously been obtained suggesting that the β subunit is synthesised before the β' subunit². Thus, if *rif* lies just to the left of the β' gene, both could be in a single operon read left to right (clockwise) on the chromosome (Fig. 1). This conclusion leads us to predict that a nonpolar deletion which begins in the *argCBH* operon and ends in the *rif* gene should place the synthesis of β' under the control of *argCBH*, which is read clockwise⁴. Expression of the *arg* genes is subject to negative control requiring at least arginine (or a derivative) and the product of the *argR* repressor gene⁸. When bacteria are grown in the presence of arginine their *argCBH* operon is fully repressed, but in its absence, the operon becomes induced and the genes in the pathway are expressed at a (ten-fold) higher rate. Still higher rates of *arg* expression (100-fold) result when cells are prevented from synthesising functional *arg* repressor by mutations in *argR*. In many *argR* mutants expression of the operon proceeds at the full constitutive level, unaffected by the presence of arginine in the growth medium⁹.

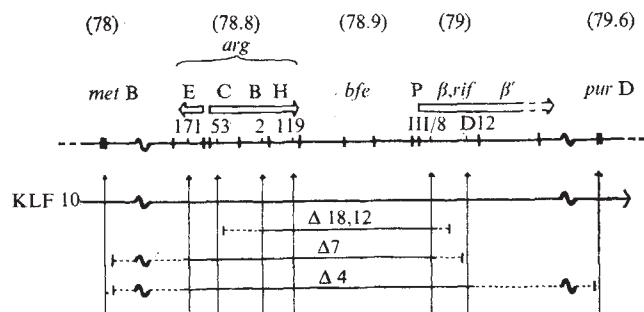


Fig. 1 The *metB-purD* segment of the *E. coli* chromosome. The figure shows the markers used in our genetic analysis, the wild-type KLF10 (omitting sex factor DNA) and the *bfe-rif* deletions isolated on this F factor. The arrows at the top of the figure indicate the direction of gene transcription and the numbers in brackets refer to the position of genes (in minutes) on the *E. coli* map²⁶.

In strains carrying KLF10 $\Delta 18$ we are able to detect changes in the overall rate of β' synthesis due to *arg* of the β' gene carried by the episome. We find that an arginine-repressed KLF10 $\Delta 18$ diploid (Table 3, line 4) synthesises nor more β' than a normal haploid strain²⁷ and markedly less than a control KLF10 diploid (Table 3, line 2). In addition, the KLF10 $\Delta 18$ diploid synthesises

significantly more β' when grown in the absence of arginine, although the rate of synthesis of β subunit is unaltered (Table 3, line 2). An isogenic control strain carrying wild-type KLF10 synthesises β and β' at a rate not affected by the presence of arginine (Table 3, lines 1 and 2). More dramatically, we find (Fig. 2 and Table 3, lines 4 and 6) that the specific increase in β' synthesis in KLF10 $\Delta 18$ strains is enlarged about seven-fold as a result of an *argR* mutation. This *argR* mutation has an equivalent effect on the synthesis of ornithine transcarbamylase, one of the *arg* enzymes (T. Bretscher and S. Baumberg, personal communication).

Measurement of the amount of β' synthesised by the KLF10 $\Delta 18$ *argR* diploid enables us to estimate the efficiency of the *argCBH* promoter, if we assume

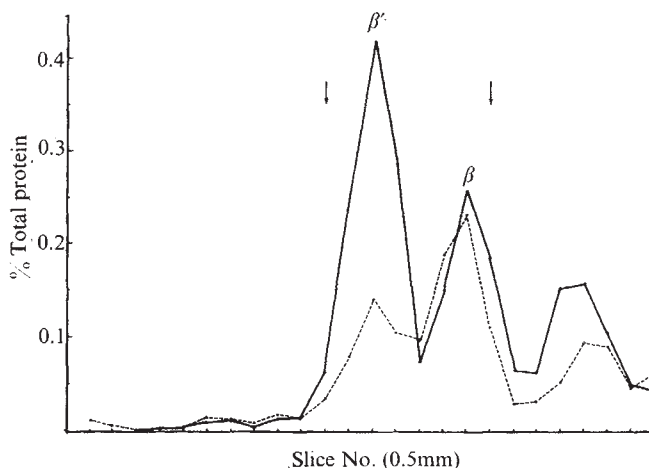


Fig. 2 Gel analysis of ³H-labelled protein in crude lysates of *argR*⁺ (LE114) and *argR*⁻ (LE117) strains carrying KLF10 $\Delta 18$. Both lysates were prepared from parallel cultures grown in excess arginine (100 μ g ml⁻¹) and run with marker polymerase on separate SDS-polyacrylamide gels (Table 1). Only the top of each gel is shown. Fractions from the *argR*⁺ and *argR*⁻ lysates are shown by broken and solid lines respectively. The limits of the stained β' and β subunits of the marker polymerase are indicated by the arrows. % Total protein = (³H per slice)/(³H in total protein applied per gel) $\times$ 100.

that $\Delta 18$ is not polar. We ascribe 0.95 (± 0.37)% of the total leucine incorporation (pulse label) to arginine-controlled β' synthesis (Table 3, lines 4 and 6). The mol% of leucine in the β' subunit (molecular weight, 160,000) is about the same as that for total *E. coli* protein¹⁰. Thus, assuming that a single cell contains 3.3×10^{-13} g of protein¹⁰, we calculate that the *argCBH* promoter is set to give about 7×10^3 – 15×10^3 protein molecules per cell. A comparable level of expression has been reported for the typtophan operon¹¹.

Control of the RNA polymerase operon

We have characterised a deletion fusing the *argCBH* operon to the middle of the *rif* gene, which puts synthesis of the β' subunit under arginine control. Our finding shows that a gene determining synthesis of the β' subunit lies to the right of *rif*. This gene, which is read clockwise, is presumably the structural gene for the β' polypeptide. The episome-coded β' synthesis we can detect in KLF10 $\Delta 18$ diploids is entirely under arginine control. The deletion $\Delta 18$ has therefore removed the normal promoter for β' synthesis without affecting the DNA sequence between *rif* and the β' gene. Consequently the promoter for β' synthesis

Table 3 Synthesis of the β and β' subunits of RNA polymerase in KLF10 diploids

Strain No.	<i>argR</i> allele	KLF10	Arginine in culture ($\mu\text{g ml}^{-1}$)	^3H in β' subunit (% total labelled protein)*	<i>P</i> †	^3H in β subunit (% in total labelled protein)*	<i>P</i> †
1 LE113	+	wild-type	None	0.59 (± 0.05)	ns	0.63 (± 0.13)	ns
2 LE113	+	wild-type	100	0.68 (± 0.06)		0.69 (± 0.09)	
3 LE114	+	$\Delta 18$	None	0.51 (± 0.08)	<0.02	0.44 (± 0.13)	ns
4 LE114	+	$\Delta 18$	100	0.37 (± 0.06)		0.45 (± 0.11)	
5 LE116	—	wild-type	100	0.57 (± 0.05)		0.84 (± 0.06)	
6 LE117	—	$\Delta 18$	100	1.32 (± 0.28)		0.56 (± 0.06)	

*Data in lines 1–4 represent the means of four lysates; those in lines 5 and 6 are means of two lysates. The standard errors of these means are shown in brackets.

†*P* values indicate the significance of the difference between cultures grown in the presence and absence of arginine computed by the method of paired comparisons²²; ns indicates the difference was not significant.

The strains used in these experiments are isogenic (Table 1). They were grown in M9 medium²³ without methionine to guard against episome loss.

Preparation of labelled cell lysates. Young M9–minimal cultures ($5 \times 10^7 \text{ ml}^{-1}$) were pulse labelled for 1.5 min with 4, 5 ^3H –leucine, chased with cold leucine for 2 min and lysed as previously described¹⁹. We used minimal medium containing $100 \mu\text{g ml}^{-1}$ arginine to grow cultures repressed for the arginine biosynthetic enzymes.

SDS polyacrylamide gel electrophoresis. We used the buffer system of King and Lemmli²⁴. The separating gel (60 mm) had 16% acrylamide, 0.094% NN'-methylene bisacrylamide (Bis), 0.025% ammonium persulphate and 0.025% (v/v), N, N, N', N'-tetramethylethylenediamine (TEMED); the stacking-gel (10 mm) had 2.5% acrylamide, 0.6% Bis and 0.1% (v/v) TEMED. It was either chemically polymerised with 0.0167% ammonium persulphate or photopolymerised with 0.0005% riboflavin. Each lysate sample (25 or 50 μl), reheated at 75°C for 5 min, was applied to the gel with bromophenol blue (final concentration 0.0002%) and marker RNA polymerase previously incubated in sample buffer²⁴. Samples were stacked at a constant current of 0.375 m per gel at room temperature for approximately 3 h and electrophoresis was continued for a further 21 h at 1.1 m per gel. Gels were stained with 0.1% Coomassie Brilliant Blue R250 in methanol:acetic acid: H_2O (5:1:5) at 37°C for 2 h and destained in 7% acetic acid/5% methanol at 37°C O/N. The β , β' region was sliced (0.5 mm) and placed in firmly capped scintillation vials containing 2 ml of Petri's permeabilising scintillation fluid²⁵ and POPOP (0.1 $\text{mg}^2 \text{ ml}^{-1}$). The vials were incubated in the dark (18 h) at 37°C . The precooled samples were counted for ^3H in a Packard liquid scintillation counter. All labelled material was eluted and the efficiency of counting was constant and reproducible. Counts were computed as a percentage of the total protein determined by counting aliquots of whole lysate under identical conditions. This method of total protein estimation gave the same results as the method of Hayward *et al.*¹³.

must lie to the left of *rif* and is presumably common to both genes.

Matzura *et al.*¹⁰ find that, under widely different conditions of growth, *E. coli* synthesises the β and β' subunits as a rather constant fraction of the total cell protein. In addition, no increase in their rate of synthesis is detected following *rif* and β' gene replication¹⁰ or in strains containing a λ *drif*⁴ transducing phage¹². Nevertheless the cell has the potential to increase in parallel the rates of β and β' synthesis to a level considerably higher than normal¹³. These combined observations indicate that the cell coordinately regulates synthesis of at least these two subunits. On the basis of the results presented here we suggest that the expression of the *rif* and β' genes is coordinately regulated at a site on the chromosome to the left of *rif*.

There are a number of candidates for a regulatory molecule acting at this site. For example, polymerase operon expression could be maintained at a constant level throughout the cell cycle if one or more of the polymerase subunits themselves acts as a repressor. An inbuilt self-regulatory function of this type has recently been attributed to operons in several organisms^{14–16}.

We are able to test whether the β' subunit alone has the regulatory function suggested. In heterodiploid strains containing KLF10 $\Delta 18$ β subunit synthesis is exclusively determined by the chromosomal *rif* gene, subject to normal polymerase operon regulation. A significant part of β' subunit synthesis, on the other hand, is regulated by arginine rising to a considerable excess (about 2.5-fold) in the *argR* strain. Thus, if the polymerase operon were self-regulated by the β' subunit, *argR*[−]/KLF10 $\Delta 18$ heterodiploids should show a concomitant reduction in the rate of β synthesis. We have been unable to find such a reduction (Table 3, lines 4 and 6) suggesting that repression of the polymerase operon is not achieved by the β' subunit acting alone at the proposed regulatory site.

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Regulation of the expression of the histidine operon in *Salmonella typhimurium*

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A positive factor enhancing the expression of this operon binds at the promoter, but exerts its effect downstream on a DNA region which acts as an attenuator of transcription.

The histidine operon of *Salmonella typhimurium* consists of nine structural genes, transcribed into a polycistronic mRNA¹⁻³ (Fig. 1). There are five unlinked regulatory genes: *hisR*, *S*, *T*, *U* and *W*¹. Products of these genes are thought to be required for the biosynthesis of a His-tRNA species involved in repression as a corepressor^{1,2,4,5}. No gene coding for an aporepressor has been found.

Although most investigators have regarded the histidine operon as a model for negative control^{1,2}, some data suggestive of positive control have appeared recently⁶. All regulatory mutations tested, including *hisR*, *S*, and *T*, and promoter-operator mutants appear to carry transcriptional regulatory defects, suggesting that the major control mechanism of the *his* operon is at the level of transcription (unpublished data of myself and P. E. Hartman). Here I report evidence of a positive regulatory mechanism required for transcription of the *his* operon and indicate the possible general importance of such positive control mechanisms.

Transcription of wild-type operon

High frequency transducing phage ($\phi 80 \lambda^{\text{imm}d\text{his}+}$) carrying the *S. typhimurium* histidine operon was isolated by Voll⁷. The regulatory behaviour of a lysogenic strain indicates that the phage retains the intact regulatory segment (G. R. Smith, B. Tong and B. N. Ames, unpublished results) as well as all the structural genes of histidine biosynthesis (Fig. 2a).

Phage DNA containing the wild type histidine operon was transcribed by purified *E. coli* RNA polymerase holoenzyme and the product was fractionated through successive hybridisation steps. After the first-step hybridisation to nontransducing phage DNA (Fig. 2a), 60–80% of the remaining ³H-mRNA had originated from the bacterial region of the transducing phage. The bacterial RNA was further hybridised to the separated strains of $\phi 80 \lambda^{\text{imm}d\text{his}+}$ DNA in the presence of excess cold RNA of various kinds (Fig. 2b). The cold competitor RNAs were from the following *Salmonella* strains: *his-515*, which is deleted for the entire bacterial region integrated into the phage genome (Fig. 2b); *his-712*, deleted for most of the histidine operon and a short distance beyond⁸; and *his01242*, a deletion of the distal portion of the operator region (Fig. 1) causing high constitutive levels of enzyme⁹. Pulse labelled *in vivo* RNA also was isolated from *his01242* cells and hybridised directly to the separated strains of $\phi 80 \lambda^{\text{imm}d\text{his}+}$ DNA as in the second step of the *in vitro* RNA fractionation (Table 1b). Results are expressed as the percentage of RNA transcribed from the bacterial segment of DNA. As Figure 2b shows the

percentage of RNA derived from the histidine operon is given as the difference between the third and fourth rows for each DNA strand in Table 1a and b.

The coding strand of the *his* operon is shown to be the *r* strand of the transducing phage DNA (45% of counts in Table 1b). Genes outside the histidine operon, possibly including the gene for gluconate-6-phosphate dehydrogenase

Table 1 Hybridisation competition *in vivo*

$\phi 80 \lambda^{\text{imm}d\text{his}+}$ RNA (³ H) hybridised the separate strands of $\phi 80 \lambda^{\text{imm}d\text{his}+}$ DNA				
a	DNA strand	Cold competitor RNA	Hybrid (³ H, c.p.m.)	%
		tRNA	2489	
		<i>his-515</i>	2258	100
	<i>r</i>	<i>his-712</i>	1407	62
				> 7
		<i>his-01242</i>	1247	55
		tRNA	1561	
		<i>his-515</i>	1535	100
		<i>his-712</i>	825	54
				> 12
		<i>his01242</i>	684	42
b	<i>his01242</i> RNA (³ H) hybridised to the separate strands of $\phi 80 \lambda^{\text{imm}d\text{his}+}$ DNA			
		tRNA	751	
		<i>his-515</i>	829	100
	<i>r</i>	<i>his-712</i>	619	75
				> 45
		<i>his01242</i>	246	30
		tRNA	312	
		<i>his-515</i>	460	100
	<i>l</i>	<i>his-712</i>	222	48
				> 5
		<i>his01242</i>	198	43

a, *In vitro* RNA was prepared as described by Nakanishi *et al.*³⁵ with some modifications. All incubations were done at 37°C. The transcription mixture contained Tris(hydroxymethyl) amino methane-HCl, pH 7.9, 20 mM, KCl 100 mM, dithiothreitol 0.1 mM, ATP, GTP, UTP, 0.15 mM each, ³H-CTP 0.075 mM (specific activity 12.5 Ci mmol, New England Nuclear Corp.), EDTA (pH 8.0) 4 mM, MgCl₂ 15 mM, rifampicin (Sigma) 10 µg ml⁻¹, $\phi 80 \lambda^{\text{imm}d\text{his}+}$ DNA 25 µg ml⁻¹ and RNA polymerase 10 µg ml⁻¹. DNA was first incubated for 2.5 min in the reaction mixture containing Tris-HCl, pH 7.9, KCl, dithiothreitol, nucleotide triphosphates and EDTA. RNA polymerase was then added and preincubation was followed for another 10 min. The transcription was started with the addition of excess MgCl₂ and was allowed to continue for 10 min. Rifampicin was added together with MgCl₂ to select for RNA chains initiated by the preinitiation complex. The total volume of the final reaction mixture was 125 µl. After the reaction was terminated by the addition of 0.5 ml of the stopping solution³⁵ containing DNase I, the reaction mixture was incubated at 0°C for 20 min. 0.5 ml of 0.1 M Tris-HCl, pH 7.2, containing 1% sodium dodecyl sulphate (SDS) and one drop of 20 mM acetic acid were added. RNA was isolated with pH 5.2 phenol and run through the double layered column of Dowex AG50W-X8 (K⁺ form) and Sephadex G-25 (ref. 36). RNA polymerase was prepared from *E. coli* N7060 cells lacking RNase I and polynucleotide phosphorylase by the method of Burgess *et al.*³⁷. The preparation had no detectable protein contamination on polyacrylamide gel electrophoresis with SDS. Cold *S. typhimurium* RNAs used in competition were isolated from exponentially growing cells. Hybridisations were carried out in two steps as described in Fig. 2, b. Each 155,000 c.p.m. (12.5 µg) of *in vivo* *his01242* ³H RNA was hybridised to the separate strands as described in Fig. 2b. Preparation of *in vivo* ³H RNA is described in Table 2.

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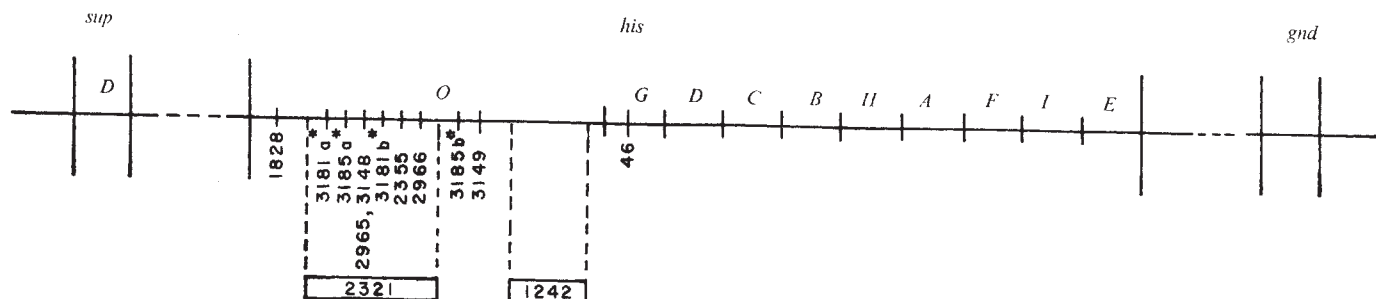


Fig. 1 The histidine operon of *Salmonella typhimurium*. The *his* operator-promoter region has been expanded, and the nine structural genes of the operon, *hisG* through *hisE*, are depicted². Two outside markers, *supD* and *gnd*, are shown. Mutation 46 is a mis-sense mutation in the *hisG* gene; all other numbers refer to operator-promoter mutations. Genetic mapping of the operator-promoter region (O), approximately 300 nucleotide pairs long, was carried out by B. Ely, D. B. Fankhauser and P. E. Hartman (unpublished results).

* It is proposed that these mutants have more than one altered site.

(*gnd* in Fig. 2), may be preferentially transcribed from the *l* strand (52% of counts from *l* strand in Table 1b). *E. coli* tRNA gave competition no greater than *his-515* RNA, indicating that the entire transcribable region of the bacterial DNA carried by transducing phage is deleted in mutant *his-515*. Compared with the high level of *in vivo* *his01242* expression, the percentage of histidine mRNA henceforth called *his* mRNA is very low when transcribed *in vitro* on $\phi 80 \lambda^{imm}his^+$ DNA as template (*r* strand in Table 1a compared with *r* strand in Table 1b). Since the level of *his* mRNA is compared with that of the neighbouring genes the results indicate that purified RNA polymerase, for some reason, does not support transcription of the wild type histidine operon to the same extent as transcription of the adjacent DNA regions.

Transcriptional barrier on DNA

Mutation *his01242* has been incorporated into the histidine transducing phage (G. R. Smith, B. Tong and B. N. Ames, unpublished results). When $\phi 80 \lambda^{imm}his01242$ DNA is used as template for purified RNA polymerase, *in vitro* expression of the histidine operon is high relative to that from the external bacterial region and is essentially the same level as that found for *in vivo* expression of *his01242* (Fig. 3a). This high rate of transcription *in vitro* is not unique for a particular RNA polymerase or DNA preparation. These results agree with the observation that *his01242* DNA supports much more synthesis of L-histidinol dehydrogenase (encoded by the *hisD* gene) than does wild type DNA in coupled and uncoupled systems of *in vitro* protein synthesis (J. Broach, S. Artz and B. N. Ames, unpublished results). Such results indicate the presence of a DNA region which lowers the expression of the histidine operon *in vitro*. Removal of this region in the deletion mutant *his01242* allows purified RNA polymerase to transcribe the histidine operon at a greatly enhanced frequency.

DNA was isolated from phage carrying the promoter-type mutation, *his02355*. Although *his02355* expression is reduced about 50-fold *in vivo* (B. Ely and Z. Ciesla, unpublished results), $\phi 80 \lambda^{imm}his02355$ DNA gives a level of *in vitro* transcription similar to wild type DNA (Fig. 3a). As expected in such experiments, the *l* strand shows low levels of hybridisation to ³H-RNA within the histidine operon (Fig. 3b). The low levels found may be the result of read-through from preceding initiation sites or abnormal initiations.

The high level of transcription of *his01242* DNA by purified RNA polymerase indicates that it is neither binding of the RNA polymerase nor RNA chain initiation which is regulated in the DNA region deleted in *his01242*. The DNA region, therefore, must either block the elongation of RNA chains in transcription or expel RNA polymerase from the

template in its travel towards the initiation site. If it is the elongation of the RNA chain that is regulated, the positive factor might act as an antiterminator.

Positive factor binds at promoter site

The genetic structure of the regulatory segment of the *his* operon has been determined (B. Ely, D. B. Fankhauser and P. E. Hartman, unpublished results) in conjunction with studies on enzyme levels (B. Ely, personal communication) and mRNA levels (unpublished data of myself and P. E. Hartman). I now focus on the most critical of these observations.

Most of the promoter-like mutations cluster upstream with respect to the *his01242* deletion (Fig. 1). The *in vivo* *his* mRNA levels of some typical promoter mutants with different genetic backgrounds are shown in Table 2. There are two kinds of promoter mutations: class I responds to the mutation *hisT1504* which produces a tRNA^{His} that is still active in protein synthesis but is not active as an effector of the *his* operon^{4,5,9,10}. Class II has lost the response to *hisT1504* or retains a very low level of response. Even though they cannot respond well to *hisT1504*, three mutations (class IIB) respond to essentially the full level of expression when they are combined as double mutants with *his01242* (last column of Table 2). It is extremely difficult to explain this phenomenon by a model exclusively involving

Table 2 Histidine mRNA levels* of promoter type mutants in different genetic backgrounds.

Strain	Type of promoter mutation	Second mutation		
		None	<i>hisT1504</i>	<i>his01242</i>
0+	Wild type	8	50	100
03181	I	8	33	44
03185	I	5	34	—
03148	IIB	10	4	98
02965	IIB	3	3	94
02355	IIA	4	5	5
02321	IIA	10	8	5
02966	IIB	8	4	92
03149	I	13	22	—

Strains were grown in the E medium of Vogel and Bonner²⁸ containing 0.1 mM L-histidine (repressed condition). Exponentially growing cells were pulse labelled for 3 min with ³H-5-uridine (New England Nuclear Corp.), and lysed with dodecylamine and SDS (ref. 39). ³H-RNA was isolated with phenol³⁶ and hybridised to denatured $\phi 80 \lambda^{imm}his^+$ DNA and $\phi 80 \lambda^{imm}DNA$ retained on Millipore filters. The relative amount of mRNA was determined from the initial slope of the *his* operon-specific DNA-RNA hybridisation curve plotted against an increasing amount of *in vivo* ³H-RNA. The methods will be described elsewhere in detail.

—, Not tested.

*The level of *his01242* was taken as 100.

The paradox is resolved by the following considerations. (1) The class IIB promoter mutations reduce the binding of a positive factor. (2) The positive factor increases the expressibility of the *his* operon in the presence of the DNA region deleted in *hisO1242*. (3) Class IIB mutants respond poorly to the *hisT1504* mutation simply because the positive factor is necessary to receive, either directly or indirectly, the altered information from the *hisT1504* mutation. Similarly, class IIB/*hisG46* (Fig. 1) double mutants grown on histidinol fail to exhibit normal physiological derepression (B. Ely, personal communication).

Attenuator: a common phenomenon ?

The existence of attenuator regions may be a general phenomenon. Extensive deletions in the *E. coli* tryptophan operon that include the region between the operator and the first cistron have a rate of transcription increased two to four-fold *in vivo* for the remaining genes, suggesting that the distal portion of the *trp* operator region limits operon expression¹¹. A transcriptional barrier has been detected on λ phage DNA¹². The *lac* operon might also contain an attenuator which is deleted or altered in UV-5 and similar mutants that exhibit, *in vivo*^{13,14} and *in vitro*¹⁵, a reduced requirement for a positive effector protein. In addition each constitutive gene has its own characteristic level of expression, and some constitutive genes are known to be susceptible to 'up-promoter' mutations¹⁶⁻²⁰. The existence of an attenuator in certain operons suggests that the potential expressibility of constitutive genes may be much higher and the determination of the constitutive level of expression may be part of the function of the attenuator. A constitutive level of gene expression could either be determined by the base sequence of the attenuator, or by the level of the positive factor. If the DNA sequence of the attenuator solely determines the level of constitutive expression, the necessity of a specific regulatory protein is eliminated.

This model proposes that not only qualitative, but also quantitative information can be coded directly by the

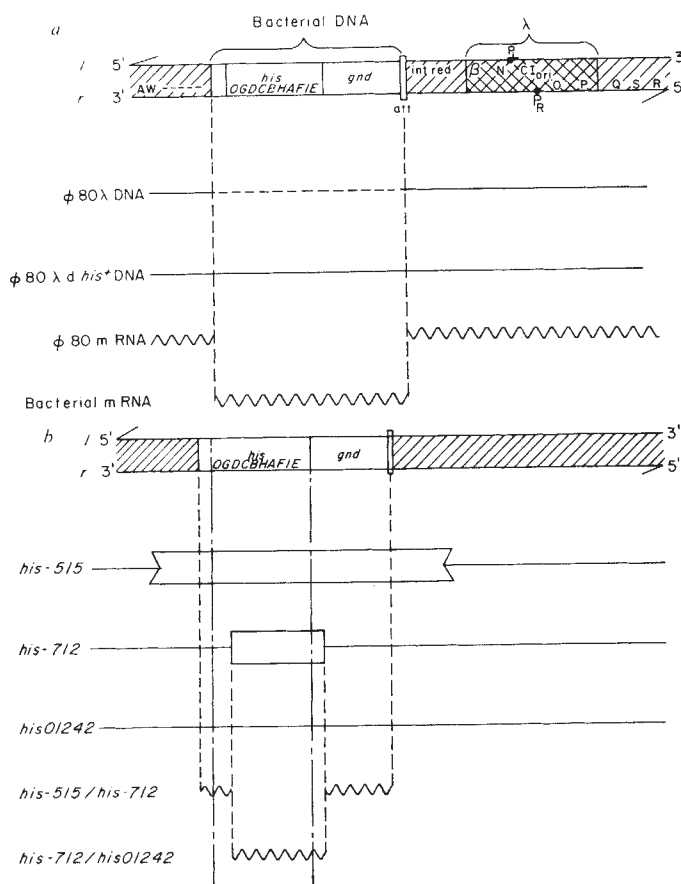


Fig. 2 Separation of histidine mRNA by two-step hybridisation. The strain used to grow the transducing phage was an *E. coli* *his* deletion mutant, doubly lysogenic for $\phi 80\lambda^{imm}dhis^{+}$ and $\phi 80\lambda^{imm}$ phages. $\phi 80\lambda^{imm}$ carries the heat-sensitive mutation, CI857. The transduced segment of bacterial DNA carries the *gnd* gene in addition to the *his* operon⁷. This segment is tentatively located to the left of *att* since there is not enough phage chromosome between *att* and *ori* to accommodate the transduced segment. The *ori* function is required for the propagation of this phage³⁰ and the lower density of the $\phi 80\lambda^{imm}dhis^{+}$ virion with respect to $\phi 80\lambda^{imm}$ (my unpublished data) indicates that the transducing phage has lost more phage DNA than is replaced by the bacterial DNA segment. Phage from heat induced lysogens were purified by polyethylene glycol precipitation³¹ and DNA was isolated from both types of phage after separation through CsCl (ref. 32). Strand separation of the transducing phage DNA was done by CsCl centrifugation in the presence of poly(U,G)³³. Unseparated DNA strands were immobilised for DNA:RNA hybridisation on Millipore nitrocellulose filters (HAWP 047 00, Millipore) according to the method of Gillespie and Spiegelman³⁴. Each filter of 6 mm diameter contained 6.7 μ g DNA. *a*, in the first step hybridisation, 90,000 c.p.m. of *in vitro* ³H-*his* was incubated with four filters which retain unseparated $\phi 80\lambda^{imm}$ DNA strands in 150 μ l of 4 $\times$ SSC (0.15 M NaCl, 15 mM sodium citrate) containing 2 mM CTP and 0.2 mg *E. coli* tRNA per ml

(General Biochemicals Co.). Cold CTP was added to chase from the filter ^3H -CTP remaining in the *in vitro* ^3H -RNA preparation. Hybridisations were carried out for 15 h at 75°C in small glass vials with screw caps. To determine the proportion of bacterial RNA in this preparation, a 25 μl sample of the first hybridisation mixture was separately transferred to a vial containing two filters of $\phi 80\lambda^{\text{imm}}\text{dhis}^+$ DNA and $\phi 80\lambda^{\text{imm}}\text{DNA}$, filled up to 50 μl with $4 \times \text{SSC}$ and incubated for 8 h at 75°C . The filters were counted as described below and the purity (P) of the bacterial RNA was determined as follows:
 $P(\%) = [\text{c.p.m. hybrid on } \phi 80\lambda^{\text{imm}}\text{dhis}^+\text{DNA} - \text{c.p.m. hybrid on } \phi 80\lambda^{\text{imm}}\text{DNA}] / \text{c.p.m. hybrid on } \phi 80\lambda^{\text{imm}}\text{dhis}^+\text{DNA}] \times 100$
b. After quenching in an ice bath, a 25 μl sample of the first hybridisation mixture was transferred to a second vial with 50 μl of $4 \times \text{SSC}$ containing $1.5\text{--}3.0 \mu\text{g ml}^{-1}$ *r* or *l* strand of $\phi 80\lambda^{\text{imm}}\text{dhis}^+\text{DNA}$ and 6 mg ml^{-1} of various cold competitor RNAs or *E. coli* tRNA. Hybridisation was continued at 74°C for and additional 7 h. At the end of the incubation, the reaction mixture was diluted with 1.5 ml of $2 \times \text{SSC}$ and the DNA-RNA hybrid was collected on a Millipore filter. After treatment with 10 μg RNase A per ml (Worthington) in $2 \times \text{SSC}$ at 37°C for 15 min, the filter was dried and counted in a toluene based scintillator.

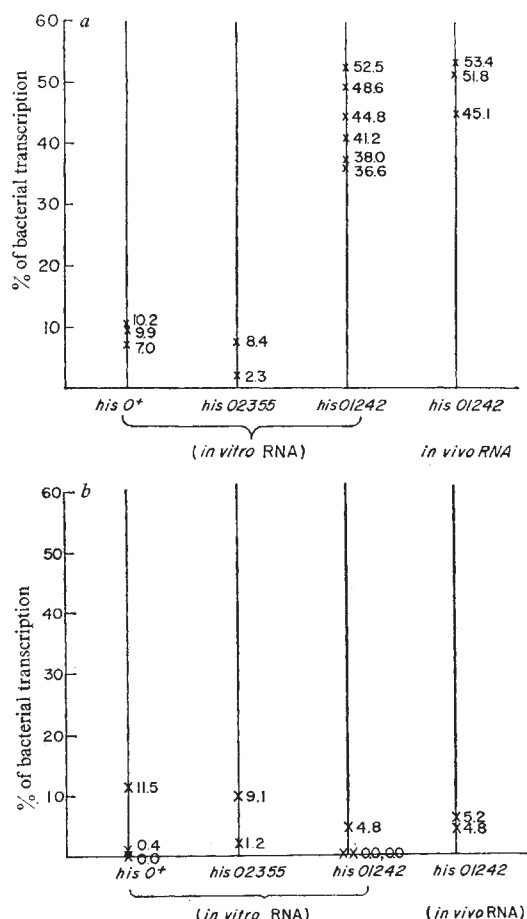


Fig. 3 Level of histidine operon transcription. Individual measurements of the fraction of *his* messenger (a) and *his* anti-messenger (b) in the RNA corresponding to the transduced bacterial segment are plotted with various templates used (*in vitro*) and with the strain *hisO1242* (*in vivo*). Methods are described in Tables 1 and 2. a, hybridisation to the *r* strand of $\phi 80\lambda^{imm}dhis^+$ DNA; b, hybridisation to the *l* strand of $\phi 80\lambda^{imm}dhis^+$ DNA

sequence of DNA. Auxiliary protein factors in transcription, positive or negative, may be regarded as means to respond to external information that is superimposed on information built into the DNA polynucleotide sequence. An alternate possibility which is unlikely but is not rigorously excluded is the following. The attenuator could be an RNA chain initiator site (*I*₁) which requires the positive factor, and *I*₁ may be followed by a cryptic RNA chain initiation site (*I*₂) which does not require positive factor. If all RNA polymerase molecules for *his* operon transcription have to travel unidirectionally from the same promoter, the attenuation can be achieved at *I*₁ by blocking an RNA polymerase molecule which is not accompanied by the positive factor. *I*₂ may be available for the RNA polymerase only when *I*₁ is deleted by a deletion such as *hisO1242*.

Control of *his* operon

I propose that expression of the histidine operon is controlled as follows. Charged His-tRNA, possibly in conjunction with a protein factor, recognises the operon-specific control site on DNA and regulates the binding of a positive factor to the promoter region. This positive factor, in turn, reduces the efficiency of an attenuator which otherwise would limit the frequency of transcription of the operon.

The function of the positive factor as a component of the transcriptional machinery suggests that this molecule is

shared by one or more classes of genes, rather than being specific to the *his* operon. Studies of regulatory mutants show that histidine enzyme levels and *his* operon transcription (unpublished results of myself and P. E. Hartman) are regulated by the concentration of His-tRNA. The action of His-tRNA could be effected through interaction with some operon-specific protein such as His-tRNA synthetase^{1,21,22} or phosphoribosyl-ATP synthetase (EC 421C)²³⁻²⁶, the target enzyme for feedback inhibition coded by the first cistron of the *his* operon²⁷⁻²⁹.

A likely DNA site for the operon-specific control is the most proximal region of the regulatory segment where a large group of constitutive mutations, such as *hisO1828* (Fig. 1) are located (B. Ely, D. B. Fankhauser and P. E. Hartman, unpublished data). The fact that a single short deletion of the *hisO1242* region gives rise to full expression makes it unlikely that any dependent negative regulatory mechanism is involved. Thus, the negative control element on the proximal region of the *his* operon probably also exerts its effect through the attenuator. Consequently, it is likely that the binding or action of the positive factor is under the control of the level of His-tRNA. This idea is supported by the observation of a requirement for positive factor-binding site for the expression of a proximally located constitutive mutation, *hisO1828*. That is, the high enzyme levels elicited by *hisO1828* are considerably decreased when it is combined in *cis* as a double mutant with a positive factor binding mutation, *hisO3148* (B. Ely, personal communication).

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UGA and non-triplet suppressor reading of the genetic code

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Mutants in a tRNA methylase gene, supK, of Salmonella typhimurium give suppression of UGA and certain frameshift mutants. In addition suppressors which do not suppress UGA, but which suppress frameshift mutants of opposite (effective) signs are described.

ADDITIONS or deletions of nucleotides in DNA in other than multiples of three are called frameshift mutations and give out-of-phase translation¹. Analysis of such mutations has shown the triplet nature of the genetic code^{2,3}, and has helped in the elucidation of the translation termination signals (or barriers) either created by mutational events within genes⁴ or naturally occurring at the ends of genes (W. P. Winter, quoted in ref. 5). Analysis of frameshift mutations has also shown the mutagenicity of various environmental chemicals⁶. We have been interested in seeing if single step mutations can give non-triplet reading at certain codons, because of the insight this may provide into the degree of fixation of the present genetic code in having three as the reading unit, and for the new types of alterations of the translation apparatus which may be revealed.

At an early stage it was found that frameshift mutations could be compensated for, or suppressed, by a second insertion/deletion change close to the site of the original mutation such that the wild type reading frame was restored^{2,4}. Suppressors of this type are termed intragenic, or internal. More recently it was found that secondary mutations external to the gene in which a frameshift mutation occurs can also specifically suppress many frameshift mutations⁷⁻¹¹. In three classes of external suppressor mutants there are alterations in the structural genes for tRNA species and another seems to have a defective tRNA-modifying enzyme¹⁵. In one mutant the frameshift suppressor tRNA has been shown to have an extra base in its anticodon¹⁶. Of four frameshift mutants which can be externally suppressed, three¹², and probably the fourth¹³, have a single base insertion in a region of single base repeats, as shown by amino acid sequence analysis of the altered protein product in intragenic (+-type) revertants. The external suppressor of at least one of these frameshift mutants acts by reading four bases, including the frameshift site, as a codon¹⁴. Non-triplet reading can also be influenced by certain mutations in the genes for two ribosomal proteins¹⁷.

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In addition to external suppressors which specifically compensate for frameshift mutations, one class of suppressors has been isolated which suppresses both a frameshift mutation, *trpA91* of *Salmonella typhimurium*⁷, and chain-terminating UGA mutations. We report that this class of suppressor mutations occurs in a gene, *supK*, which encodes a tRNA-modifying enzyme^{18,11}.

Selection of reversions

The prototype of extensively studied +1 frameshift mutants is *S. typhimurium hisD3018* (ref. 19). It has a +c insertion type frameshift mutation in the region shown in Fig. 1 (refs. 14, 20). The only external suppressors of *hisD3018* that have been characterised so far insert proline^{14,15} at a CCCU codon at the frameshift site. We decided to test whether *hisD3018* can also be suppressed by certain UGA suppressors as has been shown for *trpA91*. Strains were constructed, each containing *hisD3018*, *trpA91* and one of three different *lacUGA* mutations borne on episomes of *E. coli* origin. Spontaneous revertants selected at 30° C and 37° C in the three strains for (1) Lac⁺ (lactose utilisation) His⁺ (histidine independence), (2) Lac⁺ Trp⁺ (tryptophan independence), (3) Trp⁺ His⁺, and (4) Lac⁺ alone. At either temperature there were revertants in each category which showed phenotypic suppression of the unselected marker, and without exception all reversion to Trp⁺ His⁺ phenotypically cross-suppressed the lactose mutations.

Other combinations of mutations were tested for simultaneous reversion of the frameshift and nonsense phenotypes and did not reveal any such cross suppression, though it may be found in more extensive tests. The combinations tried were: — *hisC50* (amber)²¹ *trpA91* (FS[frameshift]); *hisC117* (ochre)²¹ *trpA91* (FS); *hisD3018* (FS) *trpA91* (FS)/F' *lac* × 82 (amber); *hisC3736* (FS)²² *trpA91* (FS)/F' *lac* UGA (three different Z alleles); *hisF3704* (FS)²² *trpA91* (FS) /F' *lac* UGA (three UGA alleles); *trpA872* (FS) *hisD3018* (FS) /F' *lac* UGA (three UGA alleles). Mutation *trpA872* is an internal suppressor of *trpA91*,⁷ and is not itself suppressed by *trpA91* external suppressors. In addition strains were constructed containing *trpA91*/F' *lac* UGA and either *hisD3749* or *hisD2565* (Fig. 1), known +c frameshift mutations¹². No cross suppressors were recovered when *hisD2565* was the histidine marker, but we were not able to obtain unambiguous results when *hisD3749* was the histidine mutation.

a, Amino acid and mRNA sequences of WT surrounding the site of the frameshift mutations.

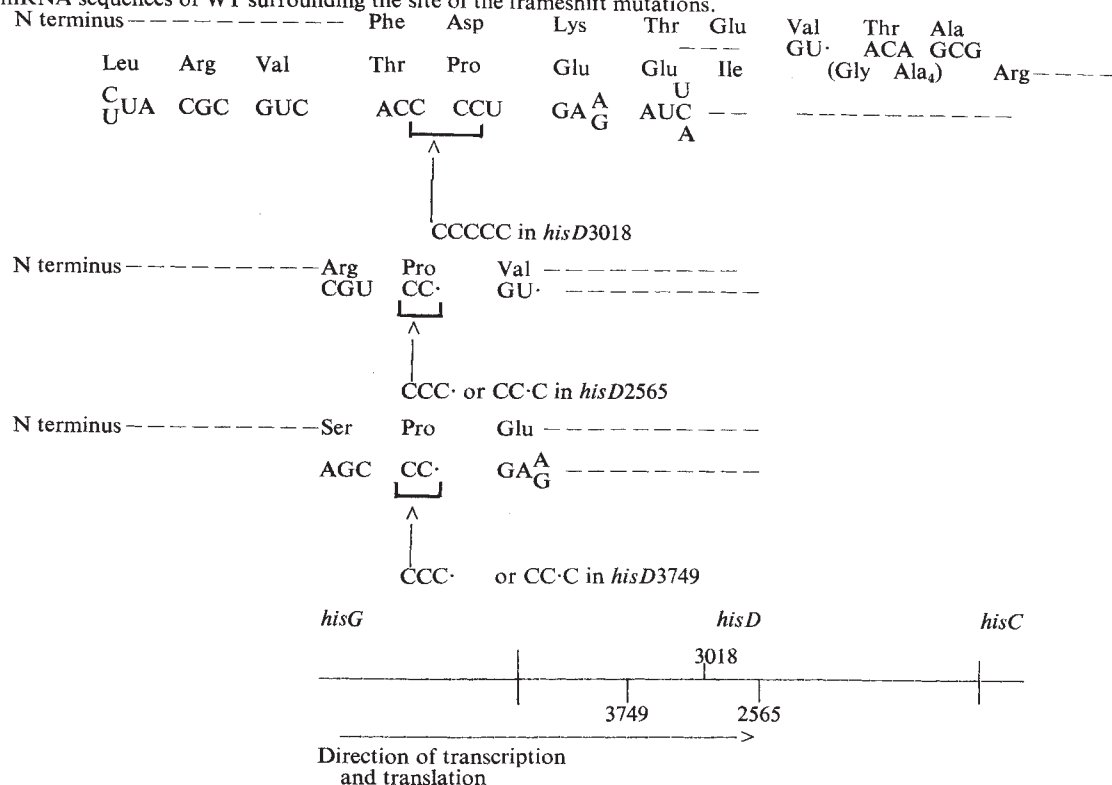


Fig. 1 The nucleotide sequences were deduced from the amino acid sequences of intragenic revertants of the frameshift mutants by Yourno and co-workers^{12,14,20}. Means any base.

Five revertants (nos 610-614) of *hisD3018trpA91/F⁺lacUGA* (Table 1) differing in temperature sensitivity and degree of cross-suppression were selected for further studies. These also had the peculiar property of different abilities to grow on nutrient agar. Since the parent strains grow well at 40° C on nutrient agar, and none of the five revertants do, we selected for back reversions at 40° C on nutrient agar. Revertants with wild type growth rate arose at a frequency of 10⁻⁸, and all had simultaneously lost the His⁺, Trp⁺ and Lac⁺ phenotypes as well as their temperature sensitivity. This strongly suggested that all four phenotypic characteristics may be due to a single suppressor-generating mutation. The five revertants (610-614) were then designated *sup*-610 to -614.

Mapping suppressors

Preliminary mapping of *sup*610 to 614 indicated a location in the *argB metK* region. The mutations were further mapped by transduction methods similar to those used previously⁷ with P22 or its variants H4 or L4 (ref. 23). The recipients were *hisD3018 trpA91 pro-438 sup⁻* (alleles 610-614) / *F⁺ pro⁺ lacUGA* and the donor phage was grown on *hisC3072 trpA91 leu-1132 serA790 lys-554 sup⁻*.

sup⁻ transductants were selected at 40° C on nutrient agar, approximately 20% of the *sup⁻* recombinants were Ser⁻ Lys⁺ and 8.5% Ser⁺ Lys⁻. Reciprocal transductions with selection for Lys⁺ and scoring for His⁺ Trp⁺ Lac⁺ and t^s were plated at 30° C and gave consistent results. The suppression and temperature sensitivity characteristics were not separable, thus confirming that in each strain a single mutation was responsible for the pleiotropy. *serA* and *lys* are not cotransducible but the suppressor gene is cotransducible with either marker. Both a dominant frameshift suppressor, *sufD* (ref. 22), and a recessive UGA suppressor, *supK* (ref. 18), have been located in this region between *serA* and *lys*. Derivatives of the *sup⁺* (that is, suppressor containing) strains were constructed which were cured of the *F⁺ pro⁺ lac⁻* episomes, and had the genotypes *hisD3018 trpA91 serA790 sup⁺* in their genomes. The strains were infected with the *E. coli* F prime KLF₁₆ carrying *serA⁺ supK⁻ sufD521 lys⁺* with selection for *ser⁺*. *sufD52* does not suppress *hisD3018* or *trpA91*. The *F⁺* ductants were all His⁻ Trp⁻ and not t^s. Curing the strains of KLF₁₆ restored the His⁺ Trp⁺ and t^s characteristic of each strain, thus demonstrating recessivity of the suppressors 610 to 614. This recessivity shows non identity with *sufD*, and is consistent with their being *supK* mutants. Identity with *supK* was confirmed when previously characterised *supK* mutants -584, -1175, and -1176 (ref. 18)

Table 1 Temperature sensitivity and phenotypic suppression characteristics of five His⁺ Trp⁺ Lac⁺ reversions

Phenotypically His ⁺ Trp ⁺ Lac ⁺ Revertants	Phenotype selected originally	Temperature of selection	Growth on nutrient agar			Phenotypic suppression of		
			30° C	37° C	40° C	Lac	His	Trp
610	Lac ⁺ Trp ⁺	30° C	Yes	No	No	+++	+	+
611	Lac ⁺ Trp ⁺	30° C	Yes	Yes	No	+++	++	+++
612	His ⁺ Trp ⁺	37° C	Yes	No	No	++	+	+
613	Lac ⁺ His ⁺	37° C	Yes	Yes	No	+++	++	±
614	Lac ⁺	30° C	No	No	No	+	+	+
Parent strains of revertants 610-614			Yes	Yes	Yes	—	—	—
<i>hisD3018 trpA91 pro-438/F⁺pro⁺lac⁻ UGA</i> (three different UGA alleles)								

Strain construction methods were analogous to those described previously⁷. The *pro* mutation was of spontaneous origin and was used to hold in the episome by omitting proline from the medium. The strains were not exposed to any mutagen. All strains were *str^s*. +++; single colonies (> 1 mm diameter) in 48 h at the optimum temperature. We estimate +++ as 5-10% efficient based on our own assays of anthranilate synthetase, and comparison with *sup*-584 (see below and ref. 18) for *lac* suppression. *F⁺ pro⁺ lac UGA* were from strains TR289, 294 and 341.

showed similar frameshift and UGA suppression characteristics when cotransduced with *serA* into *his3018trpA91* strains carrying *F' lac⁻ UGA* episome.

Models for *supK* suppression

Cross suppression between UGA and another triplet (UAA) has previously been reported^{21,25} but the suppression of UGA and certain frameshift mutations described here is novel. Can these results be explained without invoking non-triplet reading?

One model in which during translation the fidelity of triplet reading may be maintained while at the same time the effect of a frameshift mutation is suppressed is as follows. A frameshift mutation may generate several nonsense codons in the new phase in an mRNA message. Normally the ribosomes do not continue translating the message beyond the first nonsense codon unless either of two conditions are fulfilled: (1) a suitable suppressor for this first nonsense is present in the cell; (2) an internal initiator codon is available in close vicinity. This initiation may be in the neutral, that is, wild-type phase.

Fulfilment of condition (1) at the first nonsense and (2) at the encounter of the second nonsense, if this nonsense is different from the first, would result in the reversal of the effect of a frameshift mutation while maintaining triplet reading. Two polypeptide fragments are then produced.

This model can be applied to suppression of *trpA91* and *hisD3018* by the UGA suppressor, *supK* by assuming UGA as the first out of phase nonsense (see Fig. 1) and either UAA or UAG as the second encountered nonsense. Complementation of the two fragments could then give active enzyme. An alternative version of this model, which could lead to the production of overlapping fragments which might be joined into full length polypeptide chains²⁶, was discussed earlier⁷. Since in both versions of this model the amino acid sequence in the region between the frameshift and the end of the first fragment is unimportant, any amino acid inserted by a UGA suppressor is likely to be acceptable at the UGA site and lead to suppression of the frameshift mutants. We tested three alleles of *supS*, a strong dominant UGA suppressor²⁷, in partial diploid strains *trpA91hisD3018ile⁻ supS⁻ / F⁺ 13met⁺ile⁺ supS⁺* and found no suppression of either frameshift. In addition in an earlier test no internal reinitiation, as detectable by complementation with the available mutants, was found in *hisD* (ref. 28). We therefore favour models in which the suppression of frameshift mutations involves non-triplet reading.

One possibility is that the non-triplet reading occurs not at the frameshift site but at the first nonsense codon encountered thereafter in the out of phase frame, for example UGA (where · means any base). This could be ruled out if a frameshift which has an amber or ochre as the first downstream out of phase nonsense codon, is suppressed by *supK* suppressors. With the available strains *hisD3749* and *hisD2565* we were unable to test this model conclusively (see above).

Alternatively suppression of the frameshift mutants may occur by non-triplet reading close to the frameshift sites. In *supK* mutant strains tRNA is undermethylated and more than one species of tRNA may be affected. The defective modification of one species of tRNA enables it to read the triplet UGA, and of a different species may allow it to read a non-triplet as a codon. It is possible that other components of the translation machinery are undermethylated in *supK* mutants, and may be causing the non-triplet reading. This is, however, unlikely in view of the specificity of the tRNA methylases which have been examined²⁹. It is known that *hisD3018* is a +1 frameshift so presumably its suppression is by quadruplet reading. The sign of *trpA91* is unknown but the most likely possibilities are that its suppression is by quadruplet or quintuplet reading in *supK* mutant strains (see below).

We find a class of external suppressors for *trpA91* which are in a gene other than *supK* and which do not also suppress UGA. External suppressors for *trpA872* have also been isolated. These too are not in the *supK* gene and do not in addition suppress UGA. Since *trpA91* and *trpA872* are compensatory frameshift mutants⁷, one must be read as a quadruplet and the other may be read as a quintuplet, or possibly as a doublet. A doublet seems unlikely because of the amount of general misreading a doublet suppressor presumably would generate. The reading of four, twice, close to each other to correct the reading is probably only a theoretical possibility in view of the very low efficiency which would be expected. Other types of experiments may have some relevance to how this type of suppression may occur. A ribosome binding experiment gave no evidence for the nature of the adjacent base affecting triplet codon anticodon interaction³⁰. But equilibrium dialysis experiments between pentamer oligonucleotides and wild type tRNA show that wild type tRNA does have weak binding to the fifth base (ref. 31 and O. C. Uhlenbeck, personal communication) as well as to the fourth³². Presumably a change outside the anticodon loop could make this binding more efficient. This would then be analogous to the situation with the first UGA suppressor tRNA sequenced³³. But on the basis of the high degree of leakiness of UGA mutants^{34,35} compared with that found with the frameshift mutants which have been examined¹⁷, and other considerations it seems more likely that the suppressor activity is due to an insertion in the tRNA anticodon itself.

The study of frameshift mutants in bacteria was initiated when a frameshift specific mutagen, ICR191 an acridine derivative, was found for bacteria²¹. The six frameshift external suppressors which have been characterised in most detail, *sufA-F* suppress frameshift mutations which arose after ICR 191 mutagenesis²². *trpA91* was however isolated after X irradiation and *trpA872* is of spontaneous origin, and the external suppressors for *trpA91* and *trpA872* are in classes which are clearly different (S. R., unpublished) from *sufA-F*. This then raises the possibility that several additional frameshift suppressor classes can be isolated.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Narrow-band observations of galactic and extragalactic sources at 1 mm

WE report the result of a search for discrete extragalactic sources of continuum radiation near 1.2 mm. The observations were made during a run of one week on the United States National Radio Astronomy Observatory (NRAO) 11-m telescope at Kitt Peak, Arizona.

We attempted observations of a total of 90 known extragalactic objects: three were probably detected (3C84, 3C120,

3C273) and two were possibly detected (3C345, 0727–11). These five objects comprise two quasars, a Seyfert galaxy, an N galaxy and an unidentified radio source. In each case the millimetre emission represents a substantial fraction of the total power of the object, and for 3C120 and 3C345 it may well exceed the power at all other known wavelengths. We also observed a number of well known galactic regions and found millimetre emission in several of them. A detailed description of the observational techniques and discussion of some of the theoretical implications of these observations will be given elsewhere.

Table 1 Probable detections

Source	1.2 mm		Broadband*			
	Observation time (min)	$\bar{S} \pm \sigma$ (f.u.)	Present work Observation time (min)	$\bar{S} \pm \sigma$	Ade, Rather & Clegg Observation time (min)	$\bar{S} \pm \sigma$
3C84 = NGC1275	100	43 ± 11 (3.9 σ)	10	28 ± 20	70	25 ± 9.5 (2.7 σ)
3C120	70	65 ± 17 (3.8 σ)	30	33 ± 9 (3.7 σ)		
3C273	140	34 ± 8 (4.2 σ)	20	48.5 ± 44	280	22 ± 4 (5.5 σ)†
Orion IRB	20	155 ± 23 (6.7 σ)‡			130	100 ± 4 (25 σ)
Sag B2	10	162 ± 45 (3.6 σ)‡				
Sag B2N (position 1' north of Sag B2)	13	211 ± 35 (6.0 σ)‡				

* The flux densities have been calculated assuming a spectrum of the form $P(\nu) \propto \nu^2$ and an effective wavelength of 1.4 mm.

† Earlier detections of 3C273 from broadband observations were reported by Low (ref. 5).

‡ Uncorrected for the effect of an extended nebula.

Table 2 Possible detections

Source	1.2 mm		Broad band			
	Observation time (min)	$\bar{S} \pm \sigma$	Present work Observation time (min)	$\bar{S} \pm \sigma$	Ade, Rather and Clegg Observation time (min)	$\bar{S} \pm \sigma$
3C345	168	18.5 ± 7.5 (2.5 σ)			40	-7 ± 7.5
0727-11	20	201 ± 69 (2.9 σ)	7	35 ± 57		
M81 = NGC3031			20	40 ± 16 (2.5 σ)	40	15.5 ± 8
M82 = NGC3034 = 3C231	60	-12 ± 15	33	17.5 ± 15	250	22 ± 7 (3.1 σ)
OR21	40	40.5 ± 14 (2.9 σ)			140	32 ± 8 (4.0 σ)
OR21N	90	25 ± 10 (2.5 σ)				

Table 3 Objects not detected at 1.2 mm. The 2σ upper limits on the flux-densities are ≤ 200 f.u. unless denoted by + ($1 \leq 00$ f.u.), * (≤ 50 f.u.), or ** (≤ 30 f.u.)

- (a) 3C Quasars: 3C48*, 138*, 147*, 196*, 220.2*, 232*, 277.1+, 279+, 286*, 309.1*, 380*
- (b) 3C Radiogalaxies: 3C270, 274**, 348*, 405*
- (c) Seyfert galaxies: NGC1068*, 2782*, 3227*, 3516**, 4051**, 4151*, 5548+, Mark 34+, 69+, 106, 110, 124, 141, 142, 279, 290, 291+, 298+, 372, 382, 391+
- (d) N galaxies: 3C79, 135, 177, 212, 227*, 234*, 318+, 371**, 381+, 390.3*
- (e) QSOs: BSO2**, B234*, B264, B272*, B340*, B382*
- (f) High frequency radio sources: 4C39.25(Q)*, 0735+17, 1331+170(Q)*, GC1514+19*, OJ287, ON325+, OP-192, OQ172(Q)*, NRA0530, OS094(Q)**, BLLAC
- (g,h) Galaxies: NGC253, 676, 2403+, 2903, 3368, 3486+, 3521, 3623, 4192, 4303, 4472, 4526, 4535+, 4654+, 4826+, 5457+, 6946, IC1613+
- (i) Galactic sources: Oph A, Crab*

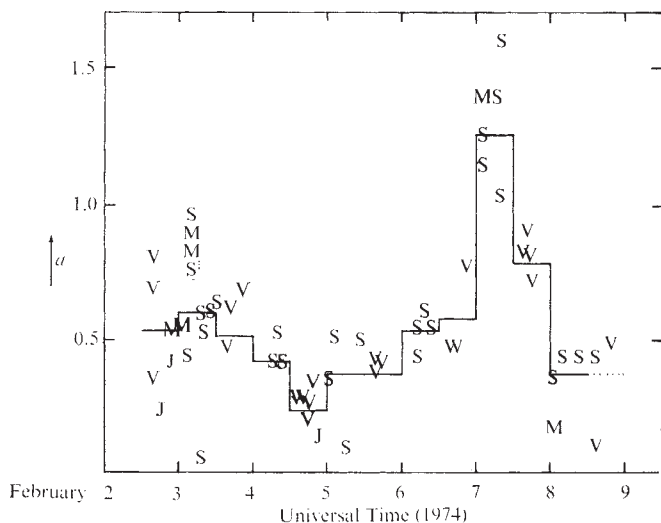


Fig. 1 Atmospheric attenuation coefficient, a , as a function of time during our observing run, from observations of the planets Venus (V), Mars (M), Saturn (S) and Jupiter (J).

In order to study as many types of object as possible, our observing list included: (a) all 3C quasars; (b) all 3C radio galaxies with $z \leq 0.02$; (c) all known Seyfert galaxies with NGC or Markarian numbers and $\delta \geq -2^\circ$; (d) all known N galaxies; (e) all QSOs with $z \leq 0.2$ in the Braccisi and Formigini list¹ of optically selected blue stellar objects; (f) all known radio sources with $|b| > 10^\circ$ satisfying one or both of the criteria $S(1 \text{ cm}) \geq 4 \text{ f.u.}$, $S(3 \text{ mm}) \geq 2 \text{ f.u.}$; (g) all galaxies brighter than 11 mag (m_{pg}) with $\delta \geq -2^\circ$; (h) all infrared galaxies with $S(10 \mu\text{m}) \geq 0.1 \text{ f.u.}$, $m_{pg} \leq 12$; (i) all galactic radio sources with $S(6 \text{ cm}) \geq 30 \text{ f.u.}$ and diameter $\leq 6'$ —a total of over 200 objects. Several experimental limitations and problems prevented us from observing more than about half of this list, namely: the location of the Sun; the inability of the telescope to look within 90° of a wind in excess of 20 mile h^{-1} (three nights of our run); intermittent unstable behaviour of the detector/amplifier system; bugs in the computer software. In spite of the resulting selection effects we believe that the sources we observed are a representative sample of the main types of known extragalactic object.

Most of the sources were observed for between 10 and 20 min. A smaller group of 25 sources, comprising the nearest objects in each of the above categories (a)–(h), together with several radio sources with strong, variable, flat-spectrum emission at high radio frequencies, were observed for between 30 min and 3 h.

We used the NRAO 1-mm continuum receiver (J. D. G. Rather, P.A.R.A., and P. E. C. to be published), beam-switched over $130''$. The radiation is detected by a three-part, scandium-doped, germanium bolometer². With its standard low pass filters, this receiver has a response extending from $800 \mu\text{m}$ to 3 mm . Imperfections in the dish surface act as an additional low pass filter; at 250 GHz , the aperture efficiency is about 12% for an unpolarised feed (the 1-mm receiver is partially polarised). The overall bandpass of the standard filters, atmosphere and telescope will be published elsewhere. Because this bandpass is so large, the mean wavelength depends strongly on the spectral index of the source³. For this reason, we used additional filters to restrict the bandwidth. With these filters, the mean wavelength should be close to 1.2 mm for any reasonable spectral index. Thus our observations are the first of extragalactic sources that can genuinely be described as giving narrow band fluxes close to 1 mm (see ref. 4 for line observations of galactic sources at 1.3 mm). For comparison, some sources were observed with only the standard broadband filtering.

We determined the flux scale for the sources, and the attenuation of the atmosphere, by making frequent observations

of the planets. Planetary fluxes were computed from the temperatures given by Rather⁹, which we believe to be correct to 10%. Venus and Jupiter had to be observed through the wall of the dome, the transmission of which we measured to be 40%. We assumed the attenuation of the atmosphere to be given by $\exp(-asec z)$ where z is the zenith distance and a is a function of time. This would be true for monochromatic observations through a plane-stratified atmosphere, and we found this simple model to be a reasonable approximation.

Figure 1 shows how a varied with time during our run, measured from 61 planetary observations taken at different elevations each day. The sudden decrease in transparency on the night of February 7 was due to the arrival of a front, accompanied by light cloud, and shows the importance of frequent calibration at these wavelengths. The solid line shows the mean values of a for 12-h intervals, which we have used in the present preliminary analysis.

The lists of our probable ($\geq 3.5\sigma$) and possible (2.5 to 3.5σ) detections are given in Tables 1 and 2. The few broadband observations we made, and the results of earlier broadband measurements by Ade, Rather and Clegg in February 1973 (previously unpublished) are also shown. The data from each of the several scans on each object were converted to a flux density and corrected by the appropriate attenuation factor, before being combined into an r.m.s. error-weighted mean. ($\bar{S} = (\sum S_i / \sigma_i^2) / \sum 1 / \sigma_i^2$ where $\sigma^{-2} = \sum 1 / \sigma_i^2$ and S_i , σ_i are the mean and standard error for an individual scan). A histogram of $\bar{S}/\sigma$ is shown in Fig. 2 for all our observations: apart from the excess of points with $\bar{S}/\sigma \geq 2.5$ the histogram agrees well with the expected Gaussian distribution, indicating that there are no gross systematic errors present. We are confident that the majority of the sources with $\bar{S} \geq 2.5\sigma$ are genuine detections.

Table 3 lists the sources from which no radiation was detected.

Schematic radio, millimetre, infrared and optical spectra have been compiled from the literature for six extragalactic objects (Fig. 3). The Parkes source 0727-11 is at present unidentified: its proximity to the galactic plane means that a galactic origin cannot be ruled out. Although discussion of these spectra must await a more thorough determination of our flux-density scale and effective wavelength, the impression is that we are seeing a similar phenomenon in most of the objects, namely a flux-density rising steeply with frequency in the millimetre region. The fact that 3C273 is the nearest known quasar (this is probably true even if its redshift is not cosmological) and that 3C120 is the nearest N galaxy (it has also been classified as a Seyfert⁷ and as an N-type Seyfert⁸) suggests that strong millimetre emission may be a common property among galaxies with active nuclei. (The flux measured from the nearest Seyfert galaxy NGC4051 was $28 \pm 12 \text{ f.u.}$, on the brink of detectability.) The corresponding millimetre luminosities cannot be determined without more reliable spectral information, but they will be of order

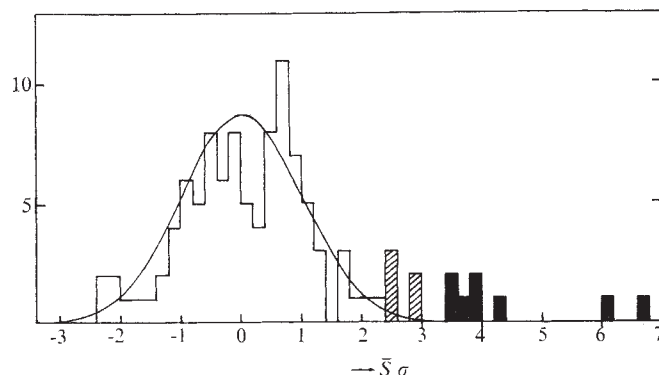


Fig. 2 Histogram of $\bar{S}/\sigma$, showing probable identifications (heavy shading), and possible identifications (light shading).

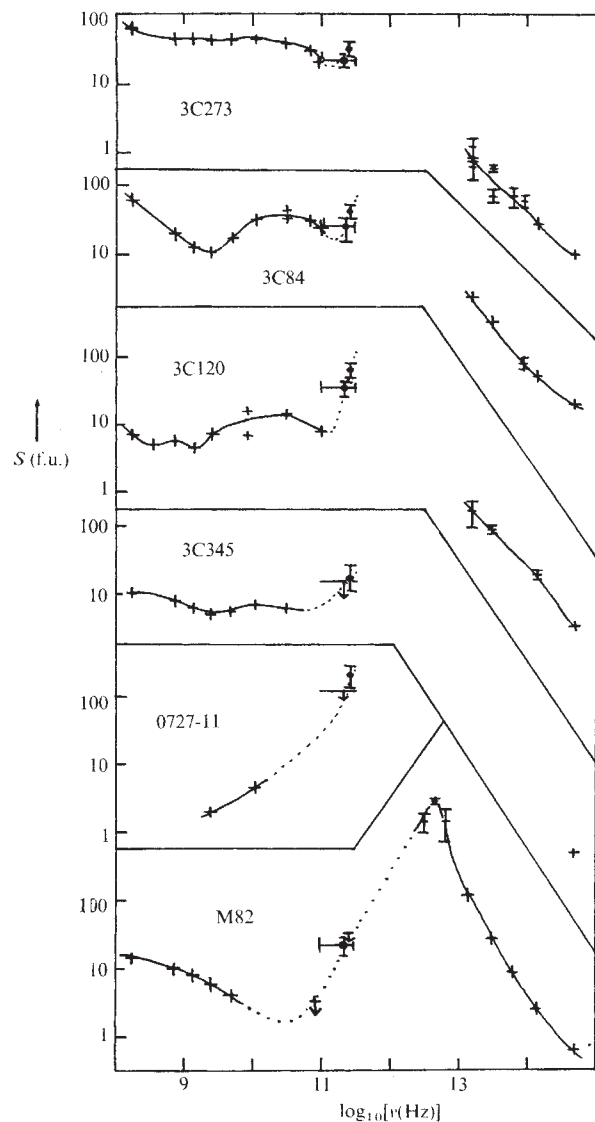


Fig. 3 Schematic radio, millimetre, infrared and optical spectra for detected extragalactic sources. Filled circle with vertical bar, present observations at 1.2 mm; circle with vertical and horizontal bars, broadband.

$P(\nu)\nu$, where $P(\nu)$ is the monochromatic luminosity, and at 1.2 mm, this quantity ranges from $\sim 10^{41}$ erg s $^{-1}$ for M82 to $\sim 10^{47}$ erg s $^{-1}$ for 3C345 if its redshift is cosmological.

The possible detection of the nearby spiral galaxy M81 is extremely surprising, since there is no other optical peculiarity which would explain why it should have a millimetre luminosity more than 10^4 times greater than our own galactic centre. This observation should be treated with caution until a less marginal detection can be achieved.

We stress that the flux densities quoted here are preliminary, although we do not expect the flux scale to be in error by more than 25%. We also emphasise that the search we have made is in no sense complete down to some flux level, owing to the strong dependence of the atmospheric attenuation on zenith distance and time. But among the objects mentioned in this paper, we doubt if we have missed any sources brighter than 200 f.u.

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Note added in proof: Reanalysis of the spectral response of the detector system suggests that the mean effective wavelength for our narrow band observations may be closer to 1.4 mm than 1.2 mm. If this is confirmed, the flux densities in Tables 1–3 need to be reduced by 25%.

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Spontaneous fission on the Ap star HR465

THERE is an anomalously high abundance of heavy elements on the Cr-Eu subgroup on the Ap star HR465 (refs 1–3). The overall abundance pattern of elements with $Z \geq 40$ indicates that the heavy elements originate from r -process nucleosynthesis³. In the r -process abundance curve there is a broad peak in the rare earth region centred around $Z \approx 65$ ($A \approx 165$). The rare earth elements on HR465, however, produce a peak centring near neodymium and samarium at $Z = 60 \sim 62$ (ref. 3); this clearly differs from the trend of the r -abundance pattern. Nor does the rare earth peak on HR465 correspond to the s -process abundance peak around barium and lanthanum ($A = 135 \sim 140$). Moreover the amount of barium on this star is too small to produce this rare earth peak by the local r -process, which begins from the s -process peak of barium and lanthanum⁴. Other explanations for the overabundance of rare earth elements on Ap stars do not seem appropriate for HR465 (ref. 1). I examine here an alternative possibility—that the rare earth anomaly on HR465 originates from asymmetric spontaneous fission.

According to the liquid drop model of fission⁵, the mass number M_H of the heavy fission fragment produced by the asymmetric spontaneous fission of nuclide (Z, A) is given by

$$M_H = 0.5(A - \bar{\nu}) + 0.045A(40.2 - (Z^2/A))^{1/2} \quad (1)$$

where Z^2/A must be less than 40.2, and $\bar{\nu}$ is the average number of prompt neutron per fission event. The $\bar{\nu}$ depends on the excitation energy of fission fragments, but it is approximately given by

$$\bar{\nu} = 0.117A - 25.73 \quad (2)$$

The numerical coefficients in equation (2) were determined by the least-squares method using the experimental values⁶ of $\bar{\nu}$ for spontaneous fission.

The nuclides with $A \geq 180$ can be produced in the Universe only by the r process. In the period of r -process cooling, delayed spontaneous fission would occur during the successive β decays. Using the value 1.78 for the surface asymmetry constant κ in the expression for the surface energy of a spherical nucleus, implies that all the nuclides in the range $A \geq 265$ ($Z \geq 96$) (ref. 7) would completely disappear in the neutron-rich region far from the β -stability line, because of delayed spontaneous fission with $\lambda_{sf} > \lambda_\beta$ (λ_x is the decay rate of the mode x). As for the nuclides with $250 \leq A \leq 265$, they become unstable against spontaneous fission faster than they become α -unstable with the approach of the nuclides to the β -stability

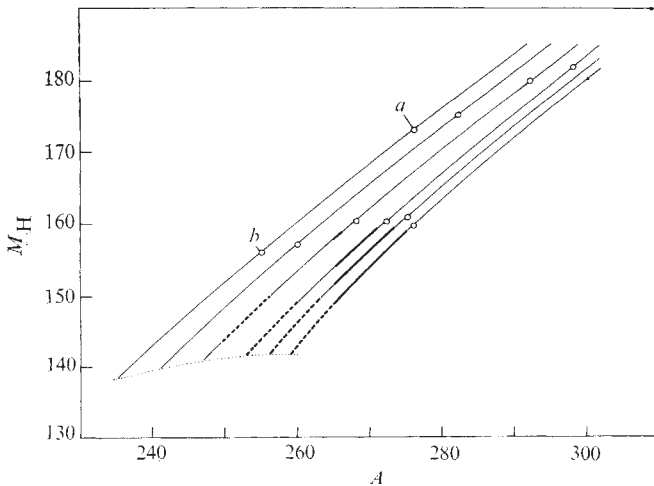


Fig. 1 Mass number M_H of the heavy fragment from the asymmetric spontaneous fission of nuclide (Z, A) . Heavy solid lines and heavy dashed lines represent, respectively, the regions for delayed spontaneous fission⁷ and regions unstable against spontaneous fission⁸. Small open circles indicate the nearest nearby nuclides to: a , $Q_n = \text{MeV}$; b , $Q_n = 4 \text{ MeV}$, β -stability line. From left to right, the six curves correspond to: $Z=92$; $Z=94$; $Z=96$; $Z=98$; $Z=99$; $Z=100$.

line ($\lambda_{sf} > \lambda_a$) (ref. 8). So they would fission completely on the neutron-rich side near the β -stability line. On the other hand, the nuclides with $A \lesssim 250$ can reach the β -stability line safely (see Fig. 1).

The β -stability line was deduced from the relation $Z = (0.3A + 100)A/(A + 200)$ (ref. 9) and the nuclides closest to $Q_n = 2$ and 4 MeV are denoted by small open circles. Here Q_n is the binding energy¹⁰ for the last neutron in the nuclide (Z, A) , and the r process usually proceeds through the neutron-rich nuclides with $Q_n = 2 \sim 4 \text{ MeV}$ (ref. 11). The maximum atomic number attainable on the r -process neutron capture path under $Q_n = 2 \sim 4 \text{ MeV}$ is $98 \sim 100$ (refs 7 and 12). The r -process cooling takes place from the neutron-rich region of $Q_n = 2 \sim 4 \text{ MeV}$ in the direction of increasing Z , keeping the mass number A constant (vertically downward in Fig. 1). If we adopt $\kappa = 1.78$, the nuclides heavier than 265 completely disintegrate by delayed spontaneous fission in the early period of the cooling to form the abundance peak of heavy fission fragments at around $A = 155 \sim 160$. Almost all the nuclides in the range $250 \lesssim A \lesssim 265$ undergo spontaneous fission in the later period of the cooling and all their fission fragments accumulate around $A = 145 \sim 150$. The Nd-Sm abundance peak on HR465 is situated at $A = 145 \sim 150$; the position of this peak just corresponds to the heavy fragment peak from the spontaneous fission of r nuclides with $250 \lesssim A \lesssim 265$. This indicates the possibility of a spontaneous fission origin for the Nd-Sm peak.

In 1960 the measured abundances of these elements on HR465 were $\text{Nd} \approx \text{Sm} \approx 10^6$ (ref. 1). I shall express the abundance of elements in a scale of $H=10^{12}$. To produce the Nd-Sm peak by the spontaneous fission of the nuclides in $250 \lesssim A \lesssim 265$, the average abundance produced, N_1 , corresponding to each mass number in the range $250 \lesssim A \lesssim 265$ must be

$$N_1 \approx 10^6 / (Y(265 - 250)) = 10^6 \quad (3)$$

where $Y (\approx 0.07)$ represents the fission yield at the heavy fragment peak when the total fission yield is set equal to 2.

The abundance of uranium on HR465 is about 3.5×10^5 (ref. 2). Although we do not have information on the age of this star, the life of main sequence Ap stars is, in general, 10^8 to 10^9 yr . The isotope ^{238}U ($4.51 \times 10^9 \text{ yr}$ half life) is, therefore, likely to survive almost completely, but the existence of ^{235}U ($7.1 \times 10^8 \text{ yr}$ half life) is not likely. The numbers of alpha-radioactive precursors for ^{238}U and ^{235}U (including those nuclei) are 3 and 6, respectively⁸. So the range of the average

abundance N_2 of the nuclides produced in the range $A \lesssim 250$ is given as follows, assuming two extreme cases of the complete survival and the complete extinction of ^{235}U :

$$3.5 \times 10^5 / (3 + 6) = 4 \times 10^4 < N_2 < 3.5 \times 10^5 / 3 = 1.2 \times 10^5 \quad (4)$$

From equations (3) and (4), the r nuclides with $250 \lesssim A \lesssim 265$ must be produced with abundance higher than that of the nuclides with $A \lesssim 250$ by a factor of 8 to 25.

The abundance peaks of the r nuclides at $A \approx 130$ and 195 correspond to the neutron magic numbers of $N=82$ and 126, respectively. The abundance of the nuclides situated on these peaks is greater than the background abundance by a factor 10 to $10^{3/2}$. Although a precise estimate of the next neutron magic number or submagic number after $N=126$ is difficult at present, $N=164$ (ref. 13) or $N=170$ (ref. 14) have been proposed. If the r -process proceeds through the path of $Q_n=3 \text{ MeV}$, the abundance (sub)peak due to the neutron (sub)magic number would be formed at around $A=250 \sim 252$ when we adopt $N=164$ as a neutron (sub)magic number, and at around $A=258 \sim 260$ when we adopt $N=170$. These peak positions were estimated by using the mass table presented by Seeger and Perisho¹⁰. In any case the r -process make an abundance (sub) peak in the region $250 \lesssim A \lesssim 265$. So the produced abundance of the nuclides in the region $250 \lesssim A \lesssim 265$ would be expected to be higher than that in $A \lesssim 250$ by about a factor 10 to $10^{3/2}$. This explains the high abundance ratio N_1/N_2 .

Thus the overabundance in the rare earth region centred near neodymium and samarium can be explained by the asymmetric spontaneous fission of r nuclides with $250 \lesssim A \lesssim 265$, which takes place in the later period of the r -process cooling. In this case the mass number $M_L (= A - \bar{\nu} - M_H)$ of the light fragment from the spontaneous fission is broadly distributed in the range $103 \lesssim M_L \lesssim 112$. Elements such as Rh, Pd, Ag, Cd and In ($Z=45 \sim 49$) are situated in this mass region, and each element is estimated to have the high abundance of at least $\sim 10^6/5 = 2 \times 10^5$. In fact the abundance of palladium on HR465 is extremely high³. The abundance determination of Rh, Ag, Cd or In is needed for the more affirmative conclusion for the spontaneous fission origin of the rare-earth peak on HR465. Furthermore we can also expect the high abundance of lead and bismuth on this star since they are the final stable elements of radioactive decay chains in $A \gtrsim 210$.

There are some peculiar A stars which have anomalously high abundances of thorium and uranium¹⁵⁻¹⁸. An abundance peak centred near neodymium and samarium similar to that on HR465 may be observed also on these Ap stars.

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Copernicus spectra of β Lyrae

WE have observed the far ultraviolet spectrum of β Lyrae using the Princeton spectrometer, on the Copernicus satellite¹. The principal data were scans in the low resolution mode (0.2 Å in the (U2) region 1,000–1,500 Å and 0.4 Å in the (V2) region 2,000–2,900 Å) at phases nearly coincident with the two light minima. Shorter scans (1,100–1,300 Å) were obtained at the two quadrature phases. All observations were made in August and September 1973 but not in the same orbital cycle.

The highest signal-to-noise ratio was obtained in the region 1,000 to 1,300 Å (Fig. 1). Beyond 1,300 Å the instrument response falls rapidly and only the strongest spectral features were detectable. In the longer wavelength region, the signal-to-noise ratio is considerably lower because of high background particle counts; here too, only major spectral features were identifiable.

The spectrum is spectacularly different from B8, the classification given in the visible region. Copernicus scans of normal stars later than about B2 show a rich absorption spectrum, particularly for those of high luminosity. The far ultraviolet spectrum of β Lyrae contains many emission lines, some with P Cygni-type profiles, some of very high excitation, but none with radial velocities associating them with the primary B8 star. It seems that the spectrum in this region is predominantly from a hotter source than the visible primary, supporting several recent suggestions^{2–5} that the secondary component in β Lyrae is the hotter object. The recently revived⁶ idea of an F-type secondary seems to be eliminated.

A few absorption lines corresponding to the B8 spectrum are observed longwards of 2,400 Å. Multiplet 1 of FeII is present completely in absorption, and estimates of its velocity indicate an amplitude (in phase with the B8 spectrum) of 340 km s⁻¹. The primary star velocity amplitude from the ground based data is 370 km s⁻¹ (ref. 7). The strongest transitions from the excited levels of SiIII and SiII are also seen in absorption in this part of the spectrum, with a velocity range of 380 km s⁻¹.

The far ultraviolet spectrum is unique among those so far observed by Copernicus in the number and strength of emission lines. Some 50 are definitely identified; an equal or greater number remains in doubt because of blending effects, or low instrumental response. Figure 1 illustrates a region of the spectrum fairly free of overlap; profiles range from classical P Cygni-type to those which resemble more a broad emission seen on both sides of a shortward-shifted central absorption. (This latter type of profile is also seen at Ha, ref. 8). These profiles are typical of a rotating, outward moving envelope with expansion velocities of ~ 150 km s⁻¹ (see refs 9 and 10). A rapidly rotating hot secondary with an outward moving envelope (in its equatorial regions), or an expanding shell around the system, is suggested.

A small velocity variation is found in the absorption and emission turning points of these profiles. In Table 1 we give the mean values derived from some 15 features which are thought to be free of blending effects. These velocity values are similar to those of the shell absorptions and emission peaks in the visible spectrum⁷ at, for example, H γ or HeI 3,889.

These numbers presumably do not represent the motion of the secondary alone, and probably are affected by mass exchange and other motions within the system.

The continuum is generally confused by overlapping emission, but its position may be estimated at 1,220, 1,150, 1,300 and 1,100 Å, in decreasing order of certainty. The flux levels, corresponding to a mean wavelength of 1,200 Å are given in Table 2.

These figures are in good agreement with the turning points of the OAO-2 light curves². They show that the quadratures have unequal light and that the secondary

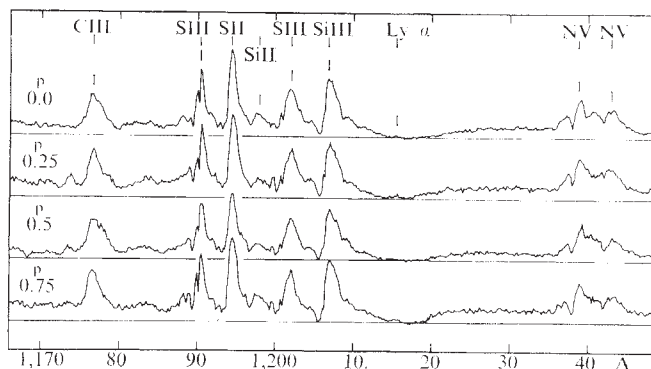


Fig. 1 Portions of Copernicus OAO spectrum scans of β Lyrae at four orbital phases.

minimum is the deeper one in the far ultraviolet. This is in keeping with the evidence that the hot radiation associated with the secondary dominates this part of the spectrum.

The emission lines do not show any marked eclipse effects. But changes are indicated in the absolute peak intensity of some lines and these are generally in the sense of being stronger than normal (by about 30%) at phase 0.0. This is comparable with the continuum changes, and evidence that the emission lines are not associated with the primary.

Table 3 summarises the strong line features identified. The Lyman lines are very broad (>10 Å), go to zero central intensity and are the only features without emission. They are similar to the Lyman lines seen in other Copernicus data and are presumably interstellar and/or circumstellar.

Helium is not conspicuous; the λ 1,085 feature may be ascribed either to HeII or a close resonance multiplet of NII. The feature is strong (peak intensity 8 to 10 times continuum) and complex, so it may be a blend.

The NII and CII lines in the V2 region showing stellar absorption velocities come from excited multiplets. The emission in the U2 region of CII and CIII (and NII if it is present at 1,085 Å) are from zero excitation multiplets.

The most important features are the NV lines, which indicate very high excitation. The presence of these lines was suspected by Houck¹¹, who found a variable emission redward of Ly α on OAO-A low resolution scans. But he favoured the interpretations at Ly α emission, because of the high excitation of NV. Our data do not display the strong phase variation of these features found by Houck; a nonperiodic change in the system may be indicated. In our data, the profiles and velocities of the NV lines differ somewhat from those of most other lines, so that the features may originate in a different part of the system.

There is no definite evidence for the presence of other lines of high excitation. No lines of NIV, CIV, OIV, or OV lie in the well observed region of the spectrum. There is an OVI line at 1,032 Å, and although a line is seen at this wavelength, there is no sign of the other component of the resonance doublet at 1,037 Å. The 1,032 Å line can be attributed to FeIII.

The Si and S lines identified are all strong features (Fig. 1). Stellar absorption components of SiII and SiIII are suspected in the V2 region. A broad emission at 1,344 Å may be the PIII (1) resonance triplet.

The spectrum of FeIII is strong and all multiplets from 1 to 59 are seen, when their lines are suitably located. The spectrum is unusual in emission in stellar spectra, and it is interesting that P Cygni itself shows FeIII emission in the visual and photographic spectral regions.

Table 1 Mean line radial velocities

Phase	0.0	0.25	0.5	0.75
Absorption (km s ⁻¹)	-175	-125	-140	-135
Emission (km s ⁻¹)	105	165	150	160

The spectrum of FeII is very rich in the observed region, but is not definitely present in our data. Lines of FeII (primary absorption) are seen in the V2 region. The presence of other iron peak ions (TiIII, IV, CrIII, MnII, III) is possible, but not certain, primarily because of blending with FeIII.

What do these observations tell us of the nature of the secondary component of β Lyrae? There is little doubt that it is a hotter object than the visible B8 star, from the presence of high temperature lines and continuum, whose velocities and intensities do not follow the behaviour of the visible primary star. The velocity amplitude of the lines suggests that the secondary is the more massive object, by a factor of several times. But, it is evident that the observed line features originate in an extended region around the secondary, which may be highly rotating, and expanding in its outer layers.

Table 2 Continuum light level at 1,200 Å

Phase	0.0	0.25	0.5	0.75
Light	0.81	1.00	0.75	1.10

The spectrum of the massive secondary is invisible in the terrestrial spectrum, either because it is shielded by the extended envelope, or because the B8 star is overluminous. The latter possibility is a strong one if the system is near the end of case B mass exchange. The system would then be rather similar to the Algol-type semidetached eclipsing binaries, and its evolutionary history would be simple. A model of this type was proposed by Kriz⁴, using the computations of Kippenhahn and Weigert¹², and more recently by J. Ziolkowski (unpublished). But overall consistency in mass, luminosity, spectral type and rate of period change, has not yet been attained.

Overluminosity of the primary, however, cannot account for all the observations. If the secondary is a BO–B2 main sequence star, as suggested by Kriz, one must assume rapid and probably differential rotation⁵. R. E. Wilson (unpublished) has interpreted the V light curve of β Lyrae using a model in which the secondary is a highly flattened rotating disk. Most of the radiation generated by the star emerges in the direction of the rotational axis, and the polar regions have the highest effective temperature, of some 11,000 to 15,000 K.

Such a temperature is insufficient to excite NV. A Copernicus survey of early-type spectra, communicated to us by D. C. Morton, shows that the NV lines are seen only in stars of type BO.5 and earlier. A model incorporating a hot spot caused by impact of mass exchange particles on the secondary, or its envelope, seems to be eliminated by the lack of strong phase dependence of the line strengths. In addition, the light curve and geometry of the mass exchange raise further objections to a hot spot hypothesis.

The differential rotation model then would require the NV lines to originate outside the orbital plane, and would also require a higher effective temperature than Wilson's model provides. An adjustment in this sense would result from introduction of a hydrogen deficiency in the primary. A similar star, KS Persei, which has a more marked hydrogen deficiency, has an apparent spectral type A5, at variance with the effective temperature 10,000 K (ref. 13). It is not clear whether such an adjustment would make Wilson's secondary hot enough.

Another serious problem is that of the reversal of eclipse depths at wavelengths below about 1,350 Å (ref. 2 and above). Wilson³ has pointed out that classical models of thermally radiating stars cannot account for this systematic variation. It is not known whether Wilson's differentially rotating model can achieve this.

An alternative representation of the system is one in which the secondary is a black hole. If matter is transferred

Table 3 Observed features

H	Ly α , β broad interstellar absorption.
HeI	Seen only at 2,829 and 2,945 Å.
HeII	Possibly present at 1,085 Å.
CII, III	Present; also primary absorption in V2 region.
NI	Possibly present at 1,085 Å; primary absorption in V2.
NV	Highest excitation found: 1,238 and 1,242 Å.
MgII	Resonance lines at 2,800 Å; P Cyg profiles.
SiII, III, IV	Well represented.
SII, III, IV	Well represented.
FeIII	Well represented; other iron peak elements?

to the vicinity of a black hole at a rate higher than $10^{-9} M_{\odot} \text{ yr}^{-1}$, an accretion disk will be formed of sufficient density to absorb X radiation from infalling material^{14,15}. This radiation then appears as a hot continuum, and radiation pressure is high enough to accelerate the excess of matter transferred over mass accreted, away from the system. They also predict the appearance of strong recombination lines of highly ionised elements, with no apparent ionisation source. The high ultraviolet luminosity also ionises the interstellar hydrogen around it, forming an extended Strömgren region. The observations described, the fact that β Lyrae is a thermal radio source¹⁶, and the mass-exchange rate of 10^{-4} to $10^{-5} M_{\odot} \text{ yr}^{-1}$, fit this picture remarkably well.

A complication of a black-hole model, which β Lyrae shares with other binary candidates such as Cygnus X-1, is its evolutionary history¹⁷. If, as is often supposed, formation of a collapsed object is associated with a supernova explosion, it is necessary to explain the present small separation and circular orbit of the components. Side stepping this point, we may sketch a possible history of the system as follows. The originally more massive star underwent extensive mass loss to its companion after its main sequence lifetime, leaving a He burning core and producing a He rich envelope on its companion. The He core should finish its evolution (as a black hole) long before the 10^6 to 10^7 yr required for the companion (the present primary) to reach the end of its main-sequence lifetime. We now see the second era of rapid mass exchange, in which the He rich layers of the present primary are transferred to an accretion disk around the collapsed companion.

A more extensive examination of the new data is being prepared. We thank the Princeton OAO staff for observing time and assistance. Copernicus is sponsored and operated by NASA.

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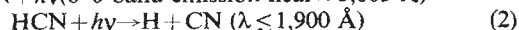
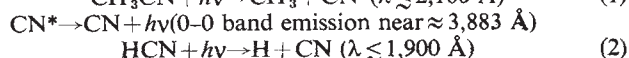
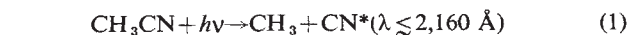
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Behaviour of Comet Kohoutek (1973f)

OBSERVATIONS of Comet Kohoutek have revealed the presence of CH_3CN (ref. 1) and HCN (W. Huebner, private communication) in the coma and of H_2O^+ in the tail²; although predicted, H_2O was not observed³.

The spectral identification of CH_3CN and HCN is the first direct support for the hypothesis first proposed by Wurm⁴, of chemically stable 'parent molecules' for the less stable radicals and ions seen in the coma and the tail. Either of these molecules could be the parent molecule of CN , which is conspicuous in almost all comets at $\lambda \approx 3,883 \text{ \AA}$. Their radiative dissociation schemes are the following^{5,6}:



These two molecules were among the earliest discovered in dense interstellar clouds⁷. This does not establish an interstellar origin for comets but seems suggestive of the environment in which comets, or more specifically their embryonic grains, condensed out of the gas phase. These two molecules are also among the most abundant products in the gas phase found in sparkings of simulated primitive planetary atmospheres⁸.

The H_2O^+ (most probably formed by photoionisation of H_2O at wavelengths shortwards of about $984 \text{ \AA}$; ref. 9); strongly suggests the presence of the latter as another parent molecule. Circumstantial evidence for the presence of H_2O as the dominant volatile component in the nucleus has been growing for some time. The strongest line of evidence has come from the recent ultraviolet and near violet observations of Comets Tago-Sato-Kosaka (1969g), Bennett (1969i), P/Encke, and now Kohoutek (1973f), all of which show strong emission from the two species $\text{H}(\text{Ly}\alpha)$ and $\text{OH}(\sim 3,040 \text{ \AA})$ (refs 10, 11 and unpublished results from J. E. Blamont and M. Festau). Detailed models of the coma computed on the basis that H and OH are the photodissociation fragments of H_2O seem to explain rather well the observed $\text{Ly}\alpha$ brightness distribution^{12,13} and the distortion of the isophotes¹⁴. Under typical cometary conditions with a large abundance of H_2O , other parent molecules would be present in the nucleus as clathrate hydrates, since these are thermodynamically more stable than their constituents¹⁵. Since the potential wells in which the 'guest molecules' are trapped in the H_2O ice lattice in the clathrate are very deep, they can be released only by the destruction of the 'host' lattice and consequently their vaporisation is controlled by the latent heat of vaporisation of H_2O . This explains the almost simultaneous appearance of all the major cometary emission bands (typically around 3 AU for most comets)

although the volatilities of the assumed parent molecules differ by more than ten orders of magnitude, provided that the abundance (by number) of the H_2O molecules exceeds all others by about six to one. This is because the clathrate lattice can typically trap only one 'guest' molecule per six 'host' H_2O molecules. The large abundance of OH ($\approx 90\%$ by number of all the radicals) observed in Comets Bester (1949k) (ref. 16) and Tago-Sato-Kosaka (1970g) (ref. 17) supports this view.

The behaviour of Comet Kohoutek cannot be explained by a pure clathrate nucleus with H_2O exceeding the other molecular species by more than 6 to 1. This is because an extensive dust halo was seen at a distance $R \approx 4 \text{ AU}$ where the vapour pressure of H_2O or the clathrate would have been negligible¹⁸. A much more volatile species is required. Carbon monoxide, which is one of the most abundant molecules discovered in interstellar space and whose ion CO^+ is probably the most abundant species in cometary tails, is a likely candidate. Formaldehyde, another abundant interstellar species which may be a 'parent' for CO according to the scheme⁹ $\text{H}_2\text{CO} + h\nu \rightarrow \text{H}_2 + \text{CO} (\lambda \lesssim 3,000 \text{ \AA})$, is also a possibility. Methane is a not unlikely alternative. Indeed, the behaviour of Comet Kohoutek can be understood in terms of a nuclear model in which the H_2O exceeds the more volatile species by a factor less than 6:1 so that a fraction of these are not trapped in the clathrate lattice. It is also likely that these more volatile species were concentrated in an outer shell because of a slow outward diffusion. As the comet approached the Sun these volatile species would quickly evaporate around $R \approx 5 \text{ AU}$, throwing out a dust halo which probably consists chiefly of clathrate hydrate grains, possibly also admixed with grains of the volatile species and more refractory, 'earthy' material. The extension (l) of this dust corona is not determined by the lifetime of the grains against evaporation at 5 AU because this would be too large for any reasonably sized grains (being about $5 \times 10^{10} \text{ s}$ for $10\text{-}\mu\text{m}$ grains). Rather it would be determined by their velocity. Taking $l \approx 10^4 \text{ km}$ and $v \approx 0.1 \text{ km s}^{-1}$ gives the time duration of mass ejection as $\approx 10^5 \text{ s}$ ($\approx 1 \text{ d}$, which seems reasonable). Assuming that the injection rate of dust is β times that of the gas (Q_{gas}) and that the dust coma is optically thin, the 'effective cross section' of the dust coma for scattering of solar radiation is

$$A_c = \beta Q_{\text{gas}} \sigma_g \Delta t / m_g = \frac{3}{4} \beta \frac{(Q_{\text{gas}} \Delta t)}{(r_g \rho_g)} \quad (3)$$

where Δt is the duration of emission of the volatiles and σ_g , r_g , m_g and ρ_g are respectively the average cross section, radius, mass and density of a grain. Since $Q_{\text{gas}} = Z_{\text{gas}} m_{\text{gas}} A_n$, where A_n is the nuclear cross section, m_{gas} is the molecular mass of the volatile and Z_{gas} is the vaporisation rate (molecules $\text{cm}^{-2} \text{s}^{-1}$), the ratio α of A_c to A_n is

$$\alpha = \frac{3}{4} \beta \frac{(Z_{\text{gas}} m_{\text{gas}})}{(r_g \rho_g)} \Delta t \quad (4)$$

Assuming that the volatile in question is CH_4 (which is representative of the highly volatile class and for which reliable data is available¹⁹), and putting $\Delta t \approx 10^5 \text{ s}$, $\beta \approx 0.1$, $r_g \approx 10 \mu\text{m}$, gives $\alpha \approx 40$. In spite of the overall uncertainty in this value arising from our guessed estimates, this shows that the 'effective cross section' at around 5 AU could have been one or two orders of magnitude larger than the nuclear cross section. Consequently, the earlier estimates of the radius could have been too large by a factor of about three to ten, accounting for the later disappointment.

If the highly volatile material involved is small, this activity will quickly cease and a much less volatile cometary nucleus consisting of clathrate hydrates will be exposed, which will then behave in a 'normal' way after the early 'anomalous' brightening

Comet Kohoutek is by no means unique in its behaviour and five out of a set of about 50 long period comets seem to show anomalous brightenings in $4 \text{ AU} \leq R \leq 5 \text{ AU}$ (ref. 20). A much larger sample (about 75% of a total of 79 comets passing perihelion in a period of 40 yr) exhibited flare activity at smaller heliocentric distances, often repeatedly^{21,22}, and indeed a significant fraction of all long period comets may be first observed during such flares²³. These flares, as well as the periodic outbursts from P/Schwassman-Wachmann 1, which moves in a nearly circular orbit with perihelion distance $\approx 5.5 \text{ AU}$, can be explained in terms of pockets of the highly volatile material contained within the clathrate lattice, being exposed to solar radiation from time to time as the overlying material evaporates. Other explanations of this phenomenon have been suggested^{24,25} but they are less direct.

Any attempt such as this to explain the differences of cometary behaviour in terms of differences in composition runs into the difficulty of having to explain why such differences in composition should exist, and this can only lead to further speculation because next to nothing is known about the spatial regions and therefore the physical and chemical environments in which comets condensed²⁶. But we require only very small differences in chemical composition to explain very different behaviour. A comet whose volatile component contains 85% H_2O molecules by number will behave in a 'normal' way, whereas one which has only 84% H_2O molecules by number will flare at a large heliocentric distance ($R \geq 5 \text{ AU}$) and then fizzle out, like Comet Kohoutek. Such small differences in chemical composition could easily be attributed to random statistical fluctuations. This may also be the difference between genuinely 'new' comets coming into the inner Solar System for the first time and those that have come in at least once before.

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Monotonic evolution of Boltzmann's H in weakly coupled gravitational systems

GASES with gravitational interactions are expected to evolve away from a state of uniform density and temperature^{1,2}. Such behaviour has been strikingly confirmed by numerical experiments on small gravitational systems³.

We point out here that this evolution does not contradict the criterion:

$$dH/dt < 0 \quad (1)$$

Indeed this criterion can even be proved. Here,

$$H = N \int d\mathbf{v} \psi(\mathbf{v}) \ln \psi(\mathbf{v}) + \text{constant} \quad (2)$$

is Boltzmann's H , ψ is the velocity distribution of particles with equal masses m . The weak coupling theory of Prigogine and Severne^{4,5} leads to a criterion of evolution of the form defined by equation 1, mainly as a byproduct of the simultaneous increase of random kinetic energy and negative correlational potential energy⁶. The criterion is illustrated in Fig. 1, which, following Miller³, is a schematic plot of the evolution of the random kinetic energy and Boltzmann's H . They do not approach a limiting value. The approach of the velocity distribution towards a Maxwellian one is deflected by an instability of the Maxwellian distributions themselves.

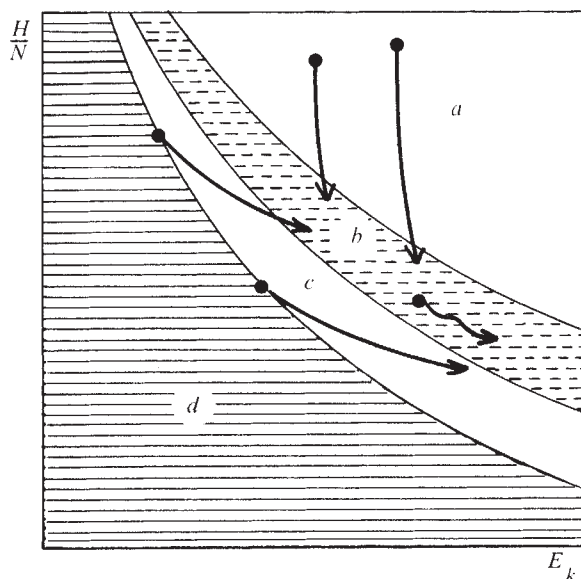


Fig. 1 The monotonic increase in mean kinetic energy per particle, E_k and the monotonic decrease in Boltzmann's H . The size of regions b and c may be much smaller than shown. The line between regions c and d represents Maxwellian velocity distributions ($2E_k dH = -3NdE_k$). Region d contains no allowed distributions. The solid circles represent hypothetical distributions at time t and the arrows show the direction of evolution of H and E_k from these points. Region a is dominated by the usual relaxation of H because of weak binary interactions. Region c is dominated by the kinetic energy production and associated negative potential energy production. The H quantity also decreases there, primarily because of increasing disorder in velocity space as a result of the heating. The evolution in region b is less rigorously established, and the region has not been shown to have boundaries independent of moments of the velocity distribution other than H and E_k . It has, however, been shown that $dE_k/dt > 0$ even in region b , and therefore $dH/dt < 0$ holds everywhere, at least over a period of time.

The Maxwellian is unstable because of distortions associated with the creation of spatial order (ref. 7, and M. J. H., not yet published). This could be crudely described as a dissipative 'flow of entropy' (and of energy) from spatial coordinates to velocity coordinates. Some local order in velocity space develops and is maintained as a byproduct, in spite of the increase in overall dispersion of the velocities.

The spatial order is not reflected in H as it is defined in equation 2, and would have to be taken into account in an appropriate definition of 'total entropy', the evolution of which could be quite different. Work is in progress on the relationship between such a total entropy and the generalised entropy concept being developed by the Brussels school.

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Sound wave modulation of auroral X rays

A UNIQUELY intense pulsation of auroral-associated X rays, followed a solar cosmic flare in September 1966 and was detected by scintillation counters on a balloon platform at an altitude of about 32 km (ref. 1). The X rays were strongly modulated with a period of 4–6 s. Figures 1 and 2 show a portion of the amplitude-modulated intensity, the ratio of intensities in two neighbouring energy channels, the power spectrum for the X-ray intensity ratio, and the spectrum of intensity variations in the lower energy channel. It can be seen that the X-ray spectrum hardens as the pulsations grow and then softens with the subsequent decay, and that there are two peaks in the X-ray power spectrum.

All these features can be interpreted as an interaction between atmospheric sound waves and a uniform flux of X rays: the intensity modulation results from an atmospheric variable density absorber formed by the sound waves, the energy dependence of the modulation derives from the energy dependence of the X-ray absorption cross section, and the two peaks in the power spectrum are possibly a result of the ducting

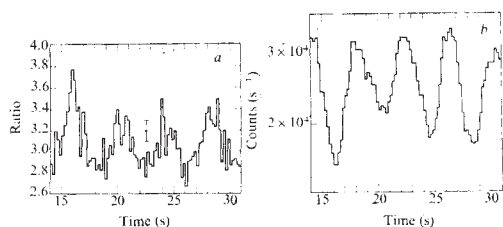


Fig. 1 *a*, Variations in the $N(25-50 \text{ keV})/N(50-73 \text{ keV})$ ratio and, *b*, $N(25-50 \text{ keV})$ intensity for four consecutive peaks in a modulated auroral X ray event, from Brown and Weir¹.

conditions for each of the atmospheric sound channels. First, however, I must digress with a description of the sound ducts.

Infrasonic ducting occurs in the lower and upper atmospheric sound channels, the lower being between altitudes of 5 and 50 km and the upper between 55 and 105 km. Figure 3 shows the lower portion of a sound speed profile based on the COSPAR model atmosphere.

Figure 4 shows the dispersion curves computed by Pfeffer and Zarichney² for some of the shorter-period, ducted, acoustic modes. An outstanding feature is the double set of plateaus, a plateau being a region of minimal dispersion—a frequency domain in which wave packets can be formed, propagate and retain identity. At the shorter periods the plateaus alternate between group speeds of 270 and 293 m s⁻¹, the sound speeds in the upper and lower ducts. The mean ratio of periods of neighbouring plateaus, averaged over the third to eighth modes, is about 1.35 (the ratio of the larger central period to the subsequent smaller central period).

The cause of the alternating plateaus, which occur when two partial ducts have a common pressure node at their interface, can be understood from the work of Tolstoy³. He shows that approximate eigenvalues for the entire duct can be represented by a lattice of points resulting from the intersections of resonant and antiresonant eigenfunctions for the partial ducts considered separately. The dispersion curve, for the solution of the complete duct, passes through a subset of the lattice points, and as the curve passes from one lattice point to a neighbouring one, most of the energy transfers from one partial duct to the other. If the ducts are excited by an impulsive source, the lattice points acquire the role of coupling frequencies which become the centre frequencies of the principal wave packets.

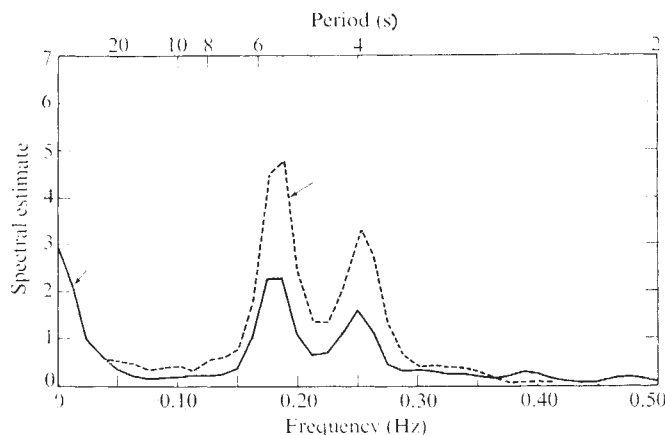


Fig. 2 Power spectra for the X ray intensity ratio $N(25-50 \text{ keV})/N(50-73 \text{ keV})$. —, Intensity variations in the energy range 25–50 keV. (From Brown and Weir¹, 1646.00 to 1649.30 UT.)

In the present case, the lattice points are an ensemble of virtual oscillators with a phase volume so severely restricted that they can be regarded as quantised in period between two levels of group speed (Fig. 4). Such an ensemble, when impulsively excited, will propagate two or more wave packets at group speeds of 270 and 293 m s⁻¹, and the power spectrum of the packets will have two or more peaks. The ratio of the frequencies of sequential peaks, as well as the ratio of the bandwidths of the peaks, is determined by the ratio of the effective widths of the partial ducts which, for the ducts shown in Fig. 3, is 1.35.

Now the ratio of the periods at which maxima occur in the X-ray spectrum is 1.38 and the ratio of the half widths of the two maxima is 22%/16%, or 1.37. These numerical correlations, between X ray and ducted sound wave spectra, can be taken as a first suggestion that the X-ray spectrum has a sonic origin. I assume that an upward-looking X-ray telescope, on a balloon platform in the lower duct, will detect modulations

of X rays by sound waves in the lower and upper partial ducts and that the spectra of the modulations will image the sonic spectra.

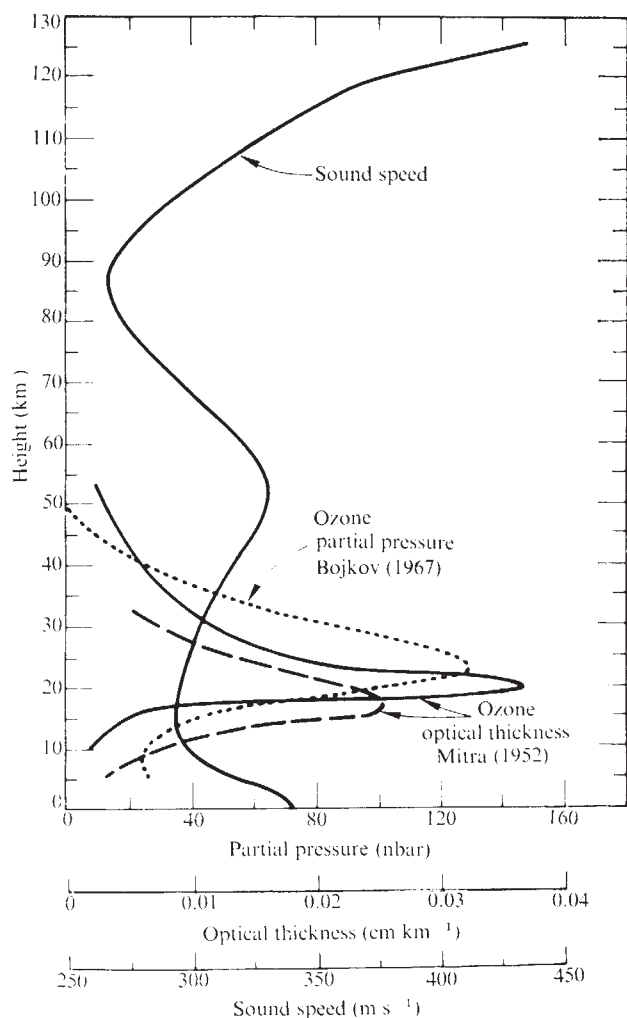


Fig. 3 Sound speed profile based on a COSPAR model atmosphere. Superimposed atmospheric ozone profiles are from Bojkov¹⁰ and Mitra¹¹.

I shall now consider the magnitude of the sonic condensation required to produce the observed X-ray modulation. Let B_0 be the average X-ray intensity at the height of observation, then the instantaneous value of the intensity, which varies due to change in absorption, will be

$$B = B_0 \exp\left(-\int_{z_0}^{z_1} \xi \mu_1 \rho dz\right) \quad (1)$$

where ρ is the ambient atmospheric density, the condensation ξ is $d\rho/\rho$, and μ_1 is the total X-ray absorption cross section in $\text{cm}^2 \text{g}^{-1}$. I take $\rho = \rho_0 \exp[-(z-z_0)/H_0]$, assume ξ and μ_1 independent of altitude and integrate equation (1) to obtain

$$\xi = -[\log B/B_0] [\mu_1 \rho_0 H_0 (1 - \exp-(z_1 - z_0)/H_0)]^{-1} \quad (2)$$

where ρ_0 and H_0 are the density and scale height at z_0 the balloon altitude, and z_1 is the height of the top of the lower duct (50 km).

To evaluate equation (2) a weighted mean cross section is the energy interval 25–50 keV must first be computed:

$$\bar{\mu}_1 = \frac{\int_{25}^{50} v \mu_1 dE}{\int_{25}^{50} v dE} \quad (3)$$

where the weighting function, $v = \exp[(25-E)/13]$, is based on the e-folding value of 13 keV observed by Brown and Weir. Using the total cross sections in nitrogen given by Siegbahn⁴, equation (3) is numerically integrated to obtain $\bar{\mu}_1 = 0.0311 \text{ cm}^2 \text{g}^{-1}$. I determine the ratio $B/B_0 = 1.25$ from the modula-

tions shown in the lower part of Fig. 1, define the rest of the terms in equation (2) for a COSPAR model atmosphere and find that the X-ray modulation would be caused by a sound wave condensation $\xi = 0.0835$; however, it is worth correcting this result for the finite angular width of the X-ray telescope, not only to obtain a more accurate condensation but to obtain internal consistency by comparing aperture efficiency with observed percentage modulation.

The aperture width is 26.5° about zenith (R. R. Brown, private communication). I use vertical and horizontal ducted wavelengths of 4.5 and 1.6 km to reconstruct the geometry of the experiment and integrate, graphically, over the solid angle of the aperture to obtain an efficiency of 57%. In making this determination, it is trivial to include a sinusoidal variation in condensation in the integrand in equation (1) because the modulation results principally from oblique alignments of like condensations, much as trees are obliquely aligned in an orchard. I include, however, an obliquity correction for a slight increase in the effective value of scale height. The computed efficiency compares well with the observed modulation, 51%, determined from the observation shown in the lower part of Fig. 1. An efficiency of 57% implies that the condensation required to produce the modulation is 14.6%.

If the ducted disturbance is regarded as the superposition of two obliquely propagating wave trains, then the required condensation in each is a half, or 7.3%. A condensation of this magnitude at 50 km altitude corresponds to 0.21% at ground level, or a pressure disturbance of 27 dyne cm^{-2} . This is surprisingly large but not unusual. Sonic disturbances of comparable magnitude at ground level have been associated with aurora for many years⁵.

Can a condensation of 14.6% also produce the energy dependence of the X-ray intensities? The variation of the ratio of the intensities in the intervals 25–50 keV and the 50–73 keV is $(d/dp) (N_1/N_2)$, or

$$d(N_1/N_2) = -(N_1/N_2) \xi \rho_0 H_0 (\bar{\mu}_1 - \bar{\mu}_2) \quad (4)$$

where $\bar{\mu}_2$ is the weighted mean cross section in the interval 50–73 keV. The negative sign indicates that the fluctuations are out of phase with the density variations, which is confirmed in Fig. 1. Taking the ratio N_1/N_2 to be the average value shown in the top of Fig. 1 and the remaining parameters as used before, I find that a 14.6% condensation will cause a variation in the ratio of X-ray intensities of $d(N_1/N_2) = 0.520$. I have again included a small obliquity correction for effective scale height. This result compares well with the directly observed value of 0.475 (in Fig. 1, half the difference between the maximum and minimum values of N_1/N_2 in the first half cycle).

One additional aspect of the X-ray event can be explained as sound wave modulation. Brown and Weir note that the first part of the event contains the 4-s peak and that the middle of the event the dominant period abruptly changes to 5.5 s. The time difference between the first and second parts of the event is $t = 105 \text{ s}$. If c_1 and c_2 are the upper and lower duct speeds, then an elementary formula, $r = c_1 c_2 t / (c_2 - c_1)$, that the interval between the two parts of the event can be attributed to the difference in propagation speeds in the two channels from a source at range 362 km.

These sound modulation calculations are also appropriate for that portion of the event which shows modulation characteristic of the upper duct, since modulation that occurs in an elevated layer is invariant to subsequent, lower level absorption. The upper duct, is however, in a more rarified part of the atmosphere and larger condensations would be required to predict the observed modulation. I am inclined to assume that energy in the upper duct extends to or just beyond the interduct boundary, and that greater condensation is not required because modulation occurs at a level near the balloon platform.

When the X-ray observations were made (September 3, 1966, Fairbanks, Alaska, latitude 65° N) the atmosphere was not appreciably different from the reference atmosphere used here. Measurements at 5, 2 and 0.4 mbar, corresponding to heights of about 32, 42 and 55 km, show that differences

between measured and reference temperatures and densities were less than 3 and 1% (ref. 6). Measured wind speeds during this part of the equinoctial period were small, between 10 and 30 knots.

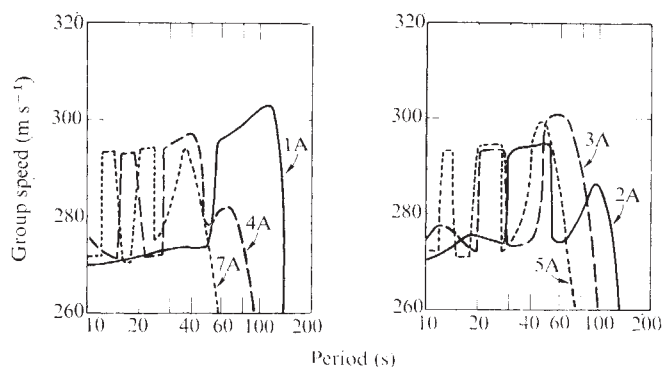


Fig. 4 Machine-computed dispersion curves for ducted sonic modes in a COSPAR model atmosphere, from Pfeffer and Zarichny². 'Plateaus' are the nearly constant speeds at peaks and troughs.

So for a single, especially clear event, it seems that a uniform X-ray flux is density-modulated by ducted atmospheric sound waves. This does not imply, of course, that the X-ray fluxes associated with aurora are always uniform, nor that all X-ray pulsations are the result of modulation by sound waves, ducted or unducted.

There is substantial evidence that ducted sound waves in the upper atmosphere result from volcanic explosions and other natural causes, as well as from nuclear detonations. Baker and Davies⁷ report the detection of wave packets generated by nuclear detonations at ranges of about 7,000 km. They conclude that the ionospheric plasma is modulated by ducted sound waves, and the plasma in turn modulates transient radio waves.

If sound waves sometimes modulate X-ray precipitations and ionospheric plasma, other atmospheric phenomena may occasionally be similarly modulated. Little attention has been given to the fluctuations which occur in atmospheric ozone density, possibly because of the use of the Dobson spectrophotometer⁸, a device in which short-term variations are averaged. Recently Hering *et al.*⁹ studied the fluctuations and concluded that a third to a half of the variance in total ozone content is a result of fluctuations in ozone density at altitudes of 11 to 15 km—the centre of the lower sound duct (Fig. 3).

I recall with pleasure conversations and correspondence with Dr Robert R. Brown.

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Five-year climatic trend in the Northern Hemisphere

RESULTS reported by Starr and Oort¹ reflect changes which have occurred in the instruments, in the methods used at the observing stations to interpret radiosonde measurements, and from the setting up of new stations, rather than from real geophysical changes.

Observations of the upper air are mainly made by measuring signals originating from airborne sensors. The measurements are converted into reports in conventional units by complex processes which incorporate corrections designed to reduce errors inherent in the system. For example, in some systems corrections are applied to the temperature measurements for the effects of lag and solar radiation on the temperature sensor, and to the pressure measurements for the effect of temperature on the pressure sensor. Different instrument systems and different conversion and correction techniques are used at different stations. Moreover, changes of instruments and techniques are introduced piecemeal. The practical effect is that, effectively, different absolute standards are brought into use. Thus, although the units are all called by the same name they have some of the properties of 'floating' international currencies in that both their international exchange rates and their apparent relation to materials vary with time. The changes are partly because of gradual improvements in the quality of techniques for observing the upper air, which are taking place at different rates in different parts of the world.

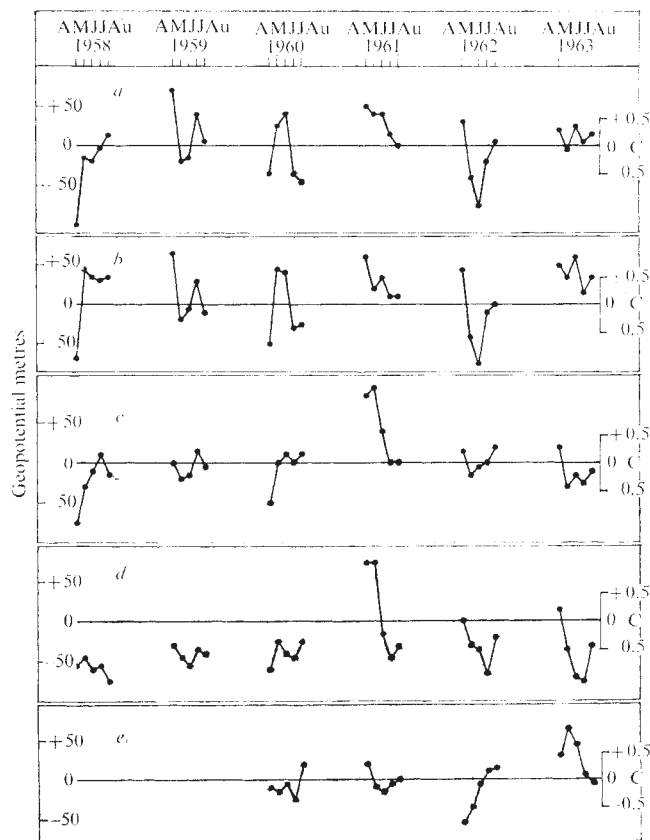


Fig. 1 Deviations of individual values of monthly mean 1,000-100 mbar geopotential thickness from 6-yr averages of the same named month from April to August. *a*, Crawley at 00Z relative to 00Z averages 1958-63; *b*, Crawley at 12Z relative to 00Z averages 1958-63; *c*, Gibraltar at 00Z relative to 00Z averages 1958-63; *d*, Gibraltar at 12Z relative to 00Z averages 1958-63; *e*, Gan at 12Z relative to 12Z averages 1960-65. Equivalent temperature scales for uniform temperature change with height are on the right. A, April; M, May; J, June; J, July; Au, August.

The most important changes have been aimed at reducing the effects of balloon wake, solar radiation and lag (in a temperature lapse environment), all of which give readings which are high relative to the true air temperature at the level. The net effect of the changes has, therefore, usually been to lower the temperature reported in conventional units relative to that which would have been formerly reported. The introduction of new stations using observing systems different from those of the pre-existing stations can have similar effects on areal analyses. World events such as the IGY 1957 and the IQSY 1964-65 tend to stimulate such changes. Water vapour mixing ratios are calculated from the measurements of pressure, temperature and relative humidity. Consequently, even if relative humidity measurements do not change, apparent changes in air temperature are associated with apparent changes in humidity mixing ratio.

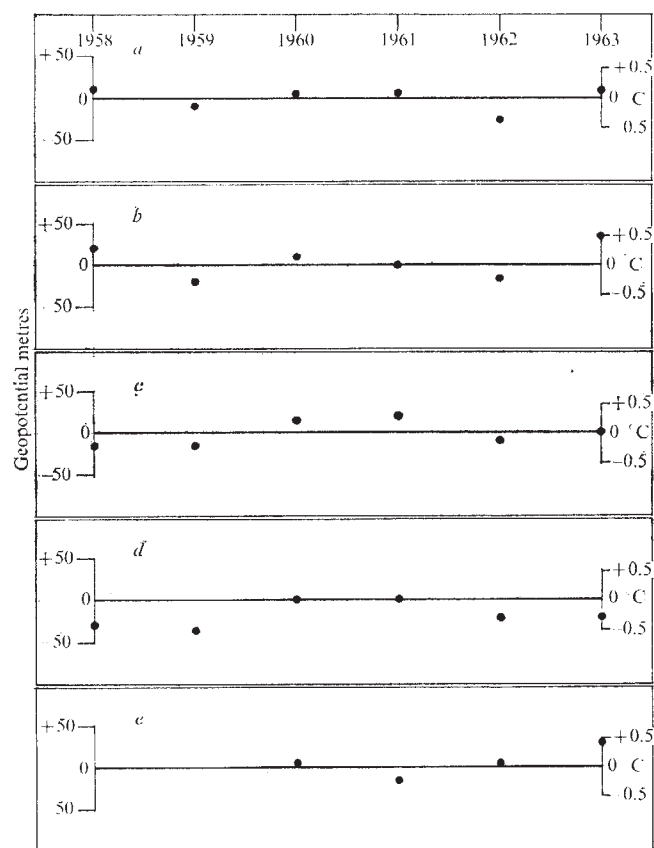


Fig. 2 Twelve-month averages, May to following April, of individual deviations of monthly mean 1,000-100 mbar geopotential thickness from the 6-yr averages of the same named months. Letters as in Fig. 1. Equivalent temperature scales for uniform temperature changes with height are on the right.

Changes such as these invalidate a straightforward geophysical interpretation¹ of the trend in temperature and humidity mixing ratio for the Northern Hemisphere.

Between 1958 and 1963 changes introduced into British radiosonde practice are likely to have had little effect upon the systematic errors of measurements from British controlled stations up to 75 mbar. Examination of quantities such as the monthly mean 1,000-100 mbar geopotential thickness for named months (preferably in summer) at such stations situated where Starr and Oort¹ (Fig. 3 of ref. 1) indicate a material trend in the vertical mean mass-weighted temperature, should serve as a direct check of the validity of a geophysical interpretation of their result. The 1,000-100 mbar geopotential thickness is a sensitive

measure of vertical mean height-weighted temperature and a decrease of 1° C in temperature, uniformly distributed in the vertical, is associated with a decrease of 67 geopotential metres (g.p.m.) in the 1,000-100 mbar geopotential thickness. The relationship between vertical mass-weighted mean temperature and 1,000-100 mbar thickness depends upon the distribution of temperature change with altitude. If, however, negative temperature changes tend to increase with altitude (as for the most part indicated by Starr and Oort, Fig. 2) a change of -1° C in the mass-weighted mean temperature would correspond to more than the -67 g.p.m. in 1,000-100 mbar thickness associated with -1° C for a uniform change of temperature with altitude.

A check has been carried out using data for individual months for Crawley (51°N 00°W), Gibraltar (36°N 5°E), and Gan (01°S 73°E). Starr and Oort¹ indicate trends close to -1° C in the vertical mean mass-weighted temperature between 1958 and 1963 at each of these stations. Each of the series showed only minor variations and no trend (Figs 1 and 2). Whether or not the last points in Fig. 2 are considered, the evidence indicates that no persistent trend in the average atmospheric temperature occurred. In both figures the mean values of 1,000-100 mbar thickness at 12Z in Gibraltar are systematically lower than associated values at 00Z. This contrasts with the result from Crawley. (If it is a real result it implies that the atmosphere over Gibraltar is a little colder near noon than near midnight.) It arises from differences on the 12Z soundings at the two stations in the mean albedo underlying the sondes. (At the time this albedo was assumed to be 0.4 for solar radiation correction techniques. When, as often happens at Gibraltar in summer, the albedo actually experienced by the sonde in 12Z soundings was less than 0.4 there was an over-correction for solar radiation effects.) It illustrates one of the ways quantities measured in the upper air and reported in the same nominal units can apparently vary in an unreal manner.

Using Gan as a new station with soundings which yield temperatures systematically lower than those of Indian radiosondes, will probably have the effect of reducing analysed temperatures within its area of influence relative to those indicated before Gan was used.

Similar apparent changes (mainly downwards) in the mean temperature of the upper air and associated changes in mean humidity-mixing ratio can be expected in some regions long after 1963.

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¹ Starr, V. P., and Oort, A. H., *Nature*, **242**, 310-313 (1973).

DRS STARR AND OORT REPLY: Hawson's contention that local changes in instruments and in the method of reducing the data would explain the downward trend in hemispheric mean temperature seems highly improbable. These factors may be important in the case of a few radiosonde stations used in the analyses. In our sample of more than 600 upper air stations, however, the instruments and procedures between different countries vary so widely that the quite systematic changes are more compatible with the interpretation of a real 5-yr trend.

Furthermore, the computed values at our lowest level (1000 mbar) agree well with those computed from surface records¹. Neither set of data shows any appreciable 5-yr temperature trend close to the surface. As the independent observations¹ were made with the aid of surface instruments which, presumably, are not affected by the radiation corrections mentioned by Hawson, the agreement suggests

Table 1 Comparison of the yearly-mean temperature deviations computed by Dronia¹ and by ourselves

	Temperature deviation from 6-yr normal for lower half of atmosphere (Dronia)						Percentage of latitude circle included	Temperature deviation from 5-yr normal for mass between surface and about 18 km (present authors). Entire latitude circle is included in computation				
	1958	1959	1960	1961	1962	1963		1958-59*	1959-60*	1960-62*	1961-62*	1962-63*
80° N	-0.14	0.76	0.36	0.26	-0.29	-0.94	100	-0.06	0.54	0.24	-0.26	-0.46
70° N	-0.22	0.38	0.38	-0.11	-0.12	-0.32	100	-0.10	0.30	0.30	-0.10	-0.40
60° N	0.01	0.11	-0.09	0.01	-0.04	0.01	100	0.06	0.06	0.16	-0.04	-0.24
50° N	0.25	-0.05	-0.35	0.25	-0.05	-0.05	89	0.14	-0.06	-0.06	0.14	-0.16
40° N	0.17	0.08	0.08	-0.02	-0.12	-0.22	56	0.22	0.02	-0.08	0.02	-0.18
30° N	0.18	0.08	0.28	-0.28	-0.12	-0.12	47	0.28	0.08	-0.02	-0.02	-0.22
20° N	0.39	0.14	0.14	-0.31	-0.21	-0.16	28	0.40	0.20	0.10	-0.20	-0.50

*May, 1958–April 1959, May, 1959–April, 1960 and so on.

that these factors do not play an important role in the upper air data which we used.

Hawson's data points, together with our values for the nearest grid point, are reproduced in Fig. 1. (For practical reasons it was not possible to extract the data at exactly the same location.) The two sets of data are in reasonably good agreement in spite of the smoothing introduced by our objective scheme of data interpolation. The trend at each grid point is evidently small in comparison with the background 'noise' from natural variability. This supports the view that significant results cannot be expected from the study of only a few months of data for a few selected points. Large areas must be considered before possible climatic trends can be delineated.

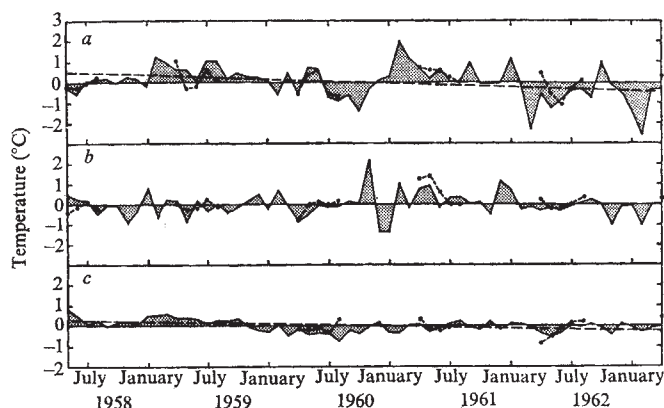


Fig. 1 Temperature deviations (00Z) from the normal annual cycle during the period May 1958–April 1963 for: a, Crawley (51.0°N, 0.1°W) trend=1.0° C every five years; b, Gibraltar (36.1°N, 5.2°W) trend=0.0° C; c, Gan (0.4°S, 73.1°E) trend=0.5° C; short-dashed line-segments, according to Hawson; full line deviations at the nearest grid points in the present analysis (52.7°N, 0.0°W; 33.9°N, 5.2°W and 0.4°S, 73.3°E, respectively); linear trend lines computed from the present data. (Our earlier map showed a trend of about -1° C every 5-yr in Gibraltar. This was because of a minor inaccuracy in the drawing of the isolines.)

Dronia² has studied a large set of mean thickness maps of the 1,000 to 500 mbar layer of about 50% of the geographical area in the Northern Hemisphere. This thickness is a measure of the vertical mean temperature in the lower half of the atmosphere. Our results are in excellent agreement with Dronia's results (Table 1) in spite of differences in data reduction and analysis procedures.

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Frictional constraints on thrust, wrench and normal faults

It is generally true that in ancient fault zones, quartzofeldspathic mylonites which indicate partial plastic yield throughout a volume of rock¹, are most extensively developed in association with thrust faults. They are less widespread along wrench faults (unless the finite displacement is very large) and are comparatively rare along normal faults². The release of seismic energy at shallow depths follows the same general pattern. More than 90% is released in areas of compressive plate interaction, mostly where underthrusting is the dominant mechanism, whereas only 6% is derived from the midocean ridges, where tensional rifting is operative³.

The analysis presented here, which accounts for these observations at least in part, quantifies a suggestion put forward by Anderson². It is applicable to any existing fault the behaviour of which is approximately governed by the linear frictional failure criterion

$$\tau > \tau_f = \mu \sigma_n$$

where τ is the applied shear stress, τ_f is the frictional shear resistance, μ is the coefficient of static friction, and σ_n is the normal stress across the fault. This will include faults made up of discrete planes and also low cohesion crush zones which can

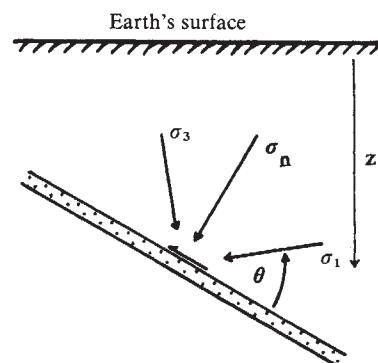


Fig. 1 Stresses around a fault zone at a depth z in the crust.

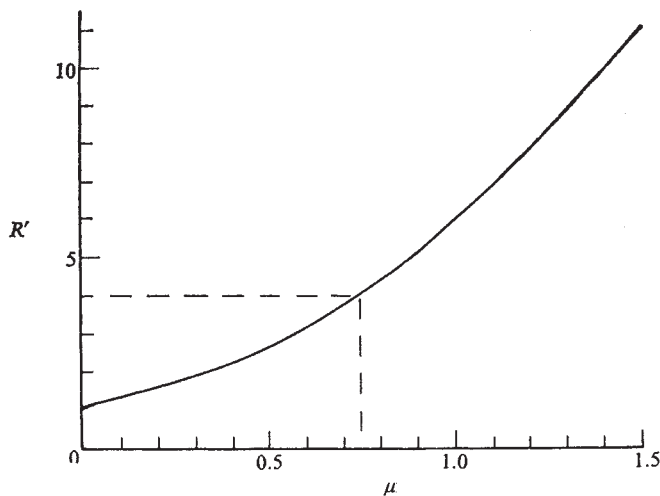


Fig. 2 Minimum ratio of principal stresses (R') required to initiate sliding on a plane with coefficient of static friction, μ .

be treated as granular aggregates of quasi-elastic rock particles⁴. (In fact, the addition of a low-value term for intrinsic cohesion across the fault will not greatly affect the outcome of this argument.) Figure 1 illustrates the stresses acting on a planar fault zone at a depth z in the crust. Throughout this discussion, σ_1 and σ_3 , the greatest and least principal compressive stresses respectively, are considered as acting in a plane normal to the

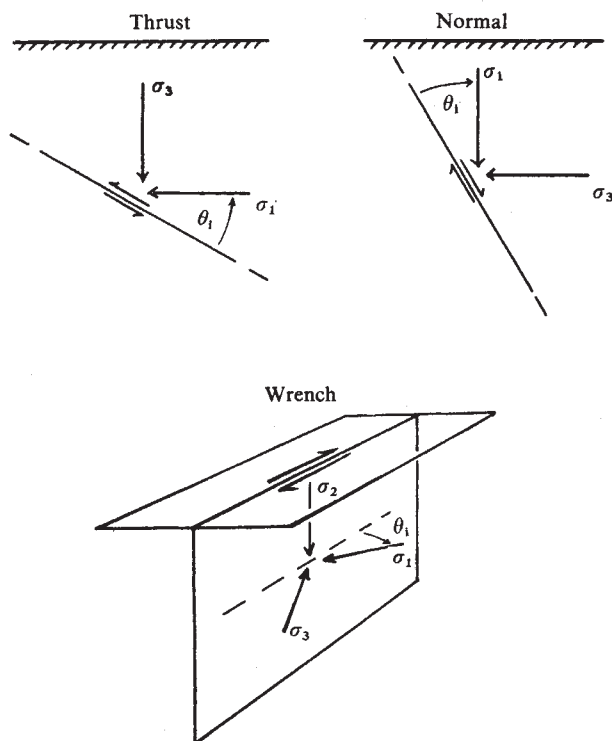


Fig. 3 Anderson's dynamic classification of faults.

fault. The condition for sliding may be rewritten in terms of the the principal stresses as

$$(\sigma_1 - \sigma_3) \sin 2\theta = \mu[(\sigma_1 + \sigma_3) - (\sigma_1 - \sigma_3) \cos 2\theta]$$

and putting $R = \sigma_1/\sigma_3$,

$$R = [\sin 2\theta + \mu(\cos 2\theta + 1)]/[\sin 2\theta + \mu(\cos 2\theta - 1)]$$

By differentiating this expression and putting $dR/d\theta = 0$, it is found that the minimum value of R required to initiate sliding is given by

$$R' = [-(1 + \mu^2) - \mu]^{-2}$$

(see Fig. 2) and occurs only when σ_1 is at an angle

$$\theta_f = \frac{1}{2} \tan^{-1}(1/\mu)$$

to the fault. At all other orientations, the ratio of principal stresses needed to initiate sliding will be greater.

Thus, in general, the limiting condition for frictional sliding is

$$\sigma_1 \geq R' \sigma_3$$

or

$$(\sigma_1 - \sigma_3) \geq (R' - 1) \sigma_3$$

Typical crustal rocks will have a static frictional coefficient, μ , of about 0.75 (refs. 4 and 5) and therefore $R' = 4$ and the corresponding value of θ_f is about 27° .

It has been shown² that many geological faults fall into three classes termed thrust, wrench and normal faults the orientation of which can be explained dynamically in terms of the Navier-Coulomb failure criterion ($\tau = \tau_0 + \mu_i \sigma_n$; where τ_0 is the cohesion and μ_i is the 'internal friction' of the rock mass), assuming that one of the principal stresses is vertical and the other two lie in a horizontal plane (Fig. 3). When the fault first forms, it does so at an angle θ_i to σ_1 , where $\theta_i = \frac{1}{2} \tan^{-1}(1/\mu_i)$. In most cases θ_i will not differ greatly from θ_f , the optimum angle for frictional sliding.

The effective overburden pressure at a depth, z , in the crust is given by

$$\sigma_v = \rho g z (1 - \lambda)$$

where ρ is the crustal density, g is the acceleration due to gravity, and the pore-fluid factor $\lambda = P/\rho g z$, P being the pore-fluid pressure.

Thus, in a thrust fault regime, where $\sigma_v = \sigma_3$, the limiting inequality for frictional sliding is

$$(\sigma_1 - \sigma_3) \geq (R' - 1) \rho g z (1 - \lambda)$$

In a wrench fault regime, $\sigma_v = \sigma_2$, and σ_2 may be written in the form

$$\sigma_2 = \sigma_3 + k(\sigma_1 - \sigma_3)$$

where $0 < k < 1$. In this case, the limiting inequality for frictional sliding is

$$(\sigma_1 - \sigma_3) \geq \{(R' - 1)/[k(R' - 1) + 1]\} \rho g z (1 - \lambda)$$

and if $\sigma_2 = (\sigma_1 + \sigma_3)/2$, (that is, $k = \frac{1}{2}$), the inequality becomes

$$(\sigma_1 - \sigma_3) \geq [2(R' - 1)/(R' + 1)] \rho g z (1 - \lambda)$$

In a normal fault regime, where $\sigma_v = \sigma_1$, the limiting inequality for frictional sliding is

$$(\sigma_1 - \sigma_3) \geq [(R' - 1)/R'] \rho g z (1 - \lambda)$$

Thus at a given depth, z , and pore-fluid factor, λ , the ratio of differential stresses required to initiate sliding on thrust, wrench ($k = \frac{1}{2}$) and normal faults is, respectively

$$(R' - 1) : 2(R' - 1)/(R' + 1) : (R' - 1)/R'$$

and if $R' = 4$ ($\mu = 0.75$), the ratio is 4:1.6:1 (Fig. 4). (Note that as k varies from 0 to 1, the minimum differential stress required for wrench faulting increases from that required for normal faulting to that needed for thrust faulting.)

The expression for the distortional elastic strain energy per unit volume can be written in the form

$$E_d = [(\sigma_1 - \sigma_3)^2/6G] (1 - k + k^2)$$

where k is as already defined and G is the rigidity modulus. Clearly, if R' is again 4, and for identical λ values, the volume density of distortional strain energy stored at the same depth around thrust, wrench and normal faults respectively (all with $k = \frac{1}{2}$) just before slip, is in the ratio 16:2.56:1. Stress regimes associated with thrust, wrench and normal faults respectively, are broadly equivalent to those inferred at crustal depths for the different types of plate boundary, arcs, transforms and ridges. Thus, the observed pattern of seismic energy release at shallow depths, and also the fact that the largest magnitude shocks occur in arc regions where underthrusting is operative, are not solely caused by varying areas of contact between lithospheric plates as has been suggested³, but are to some extent a consequence of the different stress regimes associated with the three types of plate boundary. This conclusion is of course dependent on the assumption that the shallow earthquake mechanism is indeed a process, such as stick-slip, which is controlled by friction.

Assuming that there is some upper limit to differential stress, above which it is easier for rocks to deform plastically by distributed shear throughout a volume, than by frictional sliding on a plane⁵, then the critical depth for this transition

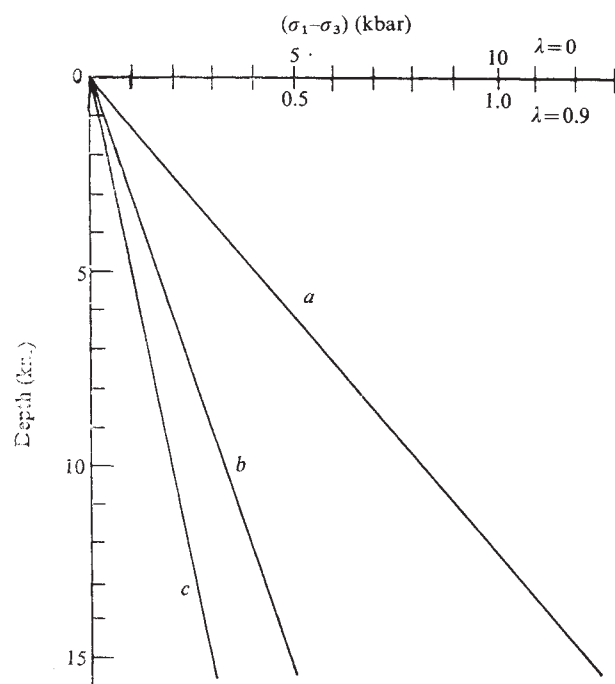


Fig. 4 Minimum differential stress required to start sliding on ideally oriented thrust (a), wrench ($k = 0.5$) (b), and normal faults (c), against depth in the crust. ($\rho = 2.8 \times 10^3 \text{ kg m}^{-3}$; $g = 9.8 \text{ m s}^{-2}$; $\mu = 0.75$; $\theta_i = 27^\circ$.)

to plastic yielding will be in the ratio 1:2.5:4 around thrust, wrench ($k = \frac{1}{2}$) and normal faults respectively, if temperature effects are neglected, $R' = 4$, and λ remains the same throughout. This explains rather well the observed distribution of mylonites in fault zones.

Absolute values for the shear stress within crustal fault zones are thought not to exceed a few hundred bars^{6,7}. It is therefore unlikely that the associated differential stress will exceed 1 kbar at the very most. If this is so, it is apparent from Fig. 4 that high pore-fluid pressures are an absolute necessity if faulting (especially thrust faulting) is to take place by frictional stick-slip to a depth of 20 km or more⁸. This condition is most likely to be maintained in the forefront of island arcs where 'wet' sediments are thought to be continually dragged down by the underthrusting process. With large wrench fault zones, it is hard to escape the conclusion that water must be continually injected into the zones from the deep crust or upper mantle, at pressures considerably greater than the hydrostatic head.

I thank Dr N. J. Price for many helpful discussions.

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Marscoite and the origin of andesites

THE present communication points out certain discrepancies between the arguments of those who dispute a contamination origin for andesitic lavas and the evidence provided by one specific and well documented example of contamination.

Green and Ringwood¹ have suggested a model for the primary derivation of andesite from mantle rocks, which relies heavily on the fact that andesites have low 'mantle-type' $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, which cannot be explained by "theories involving melting of silic material in orogenic zones, or the contamination of mantle-derived basaltic magmas with silic material". Mainly on this basis, they propose a model of mantle derivation.

In a study of Quaternary lavas from the Cascade range, Peterman *et al.*² come to a similar conclusion using two main arguments. First, that the low ratios of around 0.7032 for $^{87}\text{Sr}/^{86}\text{Sr}$ are very similar to those of the accompanying basaltic rocks and "suggest a mantle origin" (see Table 2); second, that "the extremely high Sr contents of the andesites . . . indicate that these rocks could not have been formed by . . . mixing of basaltic magma and greywacke or partial fusion of greywacke." This is seen from Table 2, and in Peterman's Fig. 1, which show that mixing of reasonable ratios of basalt and greywacke to give the major element composition of andesite would result in a rather lower Sr content for the hybrid than is actually seen in andesites.

I believe that both arguments can be answered by evidence provided by Moor bath and Bell³ in their study of strontium ratios in igneous rocks from Skye. At the classic locality of Marsco, Wager *et al.*⁴ have shown that marscoite results from the mixing at depth of a ferrodiorite magma, which they believe to be a differentiate of the Cuillin intrusion, and a porphyritic felsite magma.

Marscoite shows several analogies with the presumed manner of andesite generation by contamination theories. Both chemistry and mineralogy (Table 3) and the K-feldspar, sodic andesine, quartz and hornblende assemblage, indicate that it is an andesitic rock.

Table 1 Geochemistry of Marsco rocks

Rock type	Range of $^{87}\text{Sr}/^{86}\text{Sr}$	Average $^{87}\text{Sr}/^{86}\text{Sr}$	Average Rb (p.p.m.)	Average Sr (p.p.m.)
Ferrodiorite	0.7112–0.7133	0.7123	47	256
Marscoite	0.9124–0.7139	0.7129	78	238
Felsite	0.7123 ± 0.0020	0.7213	113	15

The most noticeable difference is that the $\text{MgO}/(\text{FeO} + \text{MgO})$ ratio is 0.173 in the marscoite and 0.443 in the hornblende andesite, this may be partly because whereas andesite, on the contamination model, is assumed to have a basaltic parent, marscoite has a more differentiated tholeiitic parent, which would be expected to impart a lower Mg/Fe ratio to a hybrid derived from it.

The acid parent of marscoite, porphyritic felsite, has a very high $^{87}\text{Sr}/^{86}\text{Sr}$ (Table 1) ratio which is typical of rocks derived by partial melting of crustal material (probably Lewisian in this case). The mixing of ferrodiorite and porphyritic felsite magmas is therefore analogous to the presumed contamination model of andesite in which basaltic magma mixes either with crustal material or with liquids produced by partial melting of such material, and as the analyses show, the Marsco case at least produces an andesitic rock. In addition, Carmichael⁶ has cited evidence of magmatic mixing in dacites from the Cascade Range, and considers that it is equivalent to contamination.

With reference to Peterman's two main arguments against the contamination origin, two statements from Moor bath and Bell are pertinent (see Table 1).

First, "a mixture of 60 parts ferrodiorite and 40 parts porphyritic felsite" (which is the ratio suggested by Wager *et al.*⁴ as necessary to give the observed major element composition of marscoite) "would have an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7127, which is still within the limit of error of both the ferrodiorite and marscoite. In spite of the fact that the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the porphyritic felsite is considerably higher than that of the ferrodiorite, the ratio of common strontium (porphyritic felsite/ferrodiorite) was presumably too low to affect the initial ratio of the ferrodiorite significantly when the rocks were mixed in the above ratio". Second, "a mixture of 60 parts ferrodiorite and 40 parts felsite would have a Sr content of 60 parts per million (p.p.m.). This is considerably lower than the value observed for marscoite and suggests that marscoite contains an excess of common strontium from some extraneous source."

The implications of these statements can be summarised thus: Marscoite shows clear evidence of being a hybrid of a basic and an acid magma, and has the same isotopic composition, within experimental error, as its basic parent; andesites, petrologically similar to marscoite, have the same isotopic composition as basalt. The hybrid origin of andesite is, therefore, not precluded by this strontium ratio data. The data on Sr concentration for marscoite and its presumed parents are inconsistent, and so these data cannot preclude a hybrid origin for andesite, particularly as the inconsistency is in the same direction for both cases.

Table 2 Geochemistry of Cascade rocks

Rock type	Range of $^{87}\text{Sr}/^{86}\text{Sr}$	Range Rb (p.p.m.)	Range Sr (p.p.m.)
Olivine basalt	0.7032–0.7039	6.6–22	200–501
Pyroxene andesite	0.7029–0.7030	31–35	1,390–1,490
Hornblende andesite	0.7032	17	1,300
Greywacke	0.705–0.708	Not given	About 200

The present analogy has its weaknesses, such as the great difference in tectonic setting between marscoite and circum-Pacific andesites, and there is not the same paucity of Sr in Peterman's greywackes relative to olivine basalt as there is in the porphyritic felsite relative to the ferrodiorite (Table 2). This is, however, somewhat countered by the simultaneously smaller difference in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the Cascade basalt and greywacke, so that the increase in Sr ratio produced by contamination is again limited, but from another quarter. A similar 60/40 mixing ratio of basalt and greywacke, each with 200 p.p.m. Sr, would produce a hybrid with a ratio around 0.705, that is, only 0.001–0.002 higher than the basalt.

In order to test this argument further, rather more precise data on the Marsco occurrence are necessary, and it may be that the accurate methods now available will allow the detection of the small (0.01%) contribution of the porphyritic felsite to the strontium ratio of marscoite.

The 'extraneous source' of strontium would seem to be the main obstacle in the path of further application of this argument. Moorbath and Bell³ state that the extraneous strontium could not have had a crustal origin, and this reflects favourably on the argument applied to the Cascade results, because it would not then be expected to alter the andesite ratio significantly, assuming that this is the reason for their high strontium contents.

Table 3 Chemical analysis of marscoite and a typical hornblende andesite

	Marscoite ³	Hornblende andesite ⁵
SiO ₂	60.07	61.5
Al ₂ O ₃	14.22	14.98
Fe ₂ O ₃	3.71	1.51
FeO	6.65	4.05
MgO	1.39	3.22
CaO	4.35	4.56
Na ₂ O	4.02	3.59
K ₂ O	2.75	2.80

Further data on other cocurrences of known hybridisation would add greatly to the understanding of this problem. In the meantime I suggest that the arguments against a contamination origin for andesite through strontium isotopic work are by no means as watertight as many papers seem to imply.

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Possible thermal explanation of contrasting Archean and Proterozoic geological regimes

THE Earth underwent a remarkable change of behaviour at the Archean-Proterozoic boundary. Greenstone belts intruded by granites dominate the geological record before about 2.5×10^9 yr ago. After that time, essentially no such greenstone belts were formed, and long linear orogens characterised by high-grade metamorphic rocks made their first appearance.

A typical Archean greenstone belt has the following characteristics. Early volcanic activity commonly ranges from ultramafic to mafic, implying high and variable degrees of partial melting of the underlying mantle. Series of volcanic rocks becoming progressively more silicic along calc-alkaline differentiation trends overlie the ultramafic flows. Clastic sedimentary rocks are sporadically interbedded with the volcanic rocks, but predominate towards the top of the pile which can reach a thickness of about 100,000 feet (ref. 1). Diapiric granite plutons cut the greenstone belts which are often preserved only as deep keels between the plutons. Structures within the greenstone belts and granitic rocks indicate that they were deformed by vertically acting forces. The rocks show only low grade metamorphism typically in the greenschist facies, although locally amphibolite and granulite grades are reached. Large areas of platformal sediments are lacking in Archean terrains.

In contrast, the Proterozoic terrains show a clear division into mobile belts and platforms. The mobile belts are long linear features characterised by high grade reworking of pre-existing rocks as well as of newly deposited rocks. Deformation is intense and complex with flow-folding predominating. Transcurrent dislocations characterise many Proterozoic mobile belts. In short, the Proterozoic mobile belts are more similar to Phanerozoic orogens than are the Archean greenstone belts.

The contrasts between the Archean and Proterozoic regimes are commonly attributed to a thin Archean sialic crust^{1,2}. But there is evidence to show that the Archean crust may have been as thick as that of the present day³. Large areas of continental crust are underlain by rocks 2.5×10^9 yr old, or older, yet today many of these areas have sialic crust of normal thickness. Either this was the Archean

thickness or sial has been added to the base of the crust since Archean times. Sial added to the base of the crust would cause isostatic rise of the continent and subsequent erosion, so that the gain in thickness of the crust would be substantially less than the amount of underplated sial. Furthermore, post-Archean isostatic uplift and subsequent erosion would expose rocks with lower K/Ar mica ages, so that Archean ages should not be preserved. We consider it much more likely that the continents have grown by lateral accretion since the Archean, possibly with their thickness being controlled essentially by sea level.

Nevertheless, the differences between Archean and Proterozoic geologic regimes outlined above require an explanation, one which is consistent with the relative sharpness and worldwide nature of the change between the two. We suggest that an explanation lies in the thermal behaviour of the Earth.

There is ample evidence to show that the Earth is cooling¹, presumably because of a decrease in heat production by radioactive decay, and also degassing to form the oceans and atmosphere. The supposedly continuous nature of this cooling has perhaps obscured its potential in causing discontinuous geologic behaviour such as the Archean-Proterozoic transition. But a simple possible explanation lies in the intersection of phase boundaries and the geotherm.

Figure 1 shows schematically the relations between geotherms which, as a result of cooling, would migrate downwards with time, and a hypothetical peridotite solidus which would be progressively higher with lower water pressure, that is, would migrate upwards because of mantle degassing. Partial melting occurs only when the geotherm and solidus intersect, and the degree of partial melting will be proportional chiefly to the excess heat involved. Thus during the Archean, with a high geothermal gradient and high P_{H_2O} , there would be melting over a wide P_{H_2O} interval, with adiabatic rise of numerous mantle diapirs probably in a random geological pattern though perhaps locally controlled by crustal fracture systems. This, along with lower pressure differentiation under high P_{H_2O} , would account

for the wide range of both extrusive and intrusive magma compositions, as well as the geological patterns of the Archean greenstone belts.

As the extent of intersection of the geotherm and the solidus is decreased as a result of cooling and mantle degassing, the degree of partial melting should decrease. One might thus expect to see a gradual change in magma compositions (a more restricted range and less hydrous nature) with time in the Archean. But as this process continues there would be a rapid change of behaviour at the point where the geotherm and solidus no longer intersect, such as at point A in Fig. 1. Magma at this point would have a very restricted composition and with further cooling melting would cease. This situation is suitable to explain the nature of the Archean-Proterozoic transition. After this condition obtained there would be no further worldwide magmatism, since it would be restricted to local areas of perturbation of the geotherm or to pockets of high water content. Geothermal perturbations could result from adjustments of the lithosphere (possibly an early form of plate tectonics), thus accounting for the linear patterns of Proterozoic magmatism and tectonics, or possibly in zones of concentrated heat flux (deep mantle plumes) for which there seems to be little Proterozoic evidence. We suggest the former process and further suggest this as an explanation for the relatively constant tholeiitic composition of Proterozoic magmas.

This paper arose from discussion with J. G. Thurlow, S. Swinden, and P. L. Dean.

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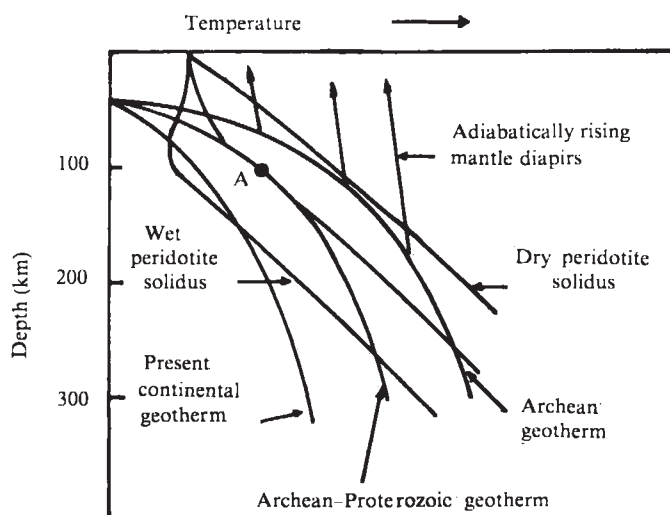


Fig. 1 Schematic relations between peridotite solidus at different water pressures, and geotherms at different times in a cooling Earth. Designed to show the nature of these effects but not precise temperatures or water pressures. See text for further explanation. (Modelled after Fig. 4 of ref. 5).

Pseudo-holography using scattered X rays

A MAJOR obstacle to the formation of images with scattered X rays is the lack of a suitable X-ray lens. Radiation at such short wavelengths is not appreciably refracted by matter, which means that a lens, in the optical sense of the word, cannot exist. To overcome this difficulty, a concept can be applied which has previously been used to form an image of sources which emit their own radiation rather than scattering radiation from another source. Basically, the concept consists of a two-step process in which the radiation is used to cast shadows of a coded aperture which are recorded on film. The encoded image is then decoded by one of several possible methods, depending on the type of aperture used. This technique was first proposed for use in X-ray astronomy¹ and has been applied to imaging distribution of γ emitters (refs 2-5, and H. H. Barrett, and F. A. Horrigan, not yet published). We have used a Fresnel zone plate as a coded aperture. This is by no means the only possible aperture, but it does offer some advantages over other types. The advantages involve primarily the ease with which decoding can be accomplished.

The Fresnel zone aperture consists of concentric annular zones of equal area which are alternately transparent and opaque to X rays. Figure 1 illustrates its operation. A

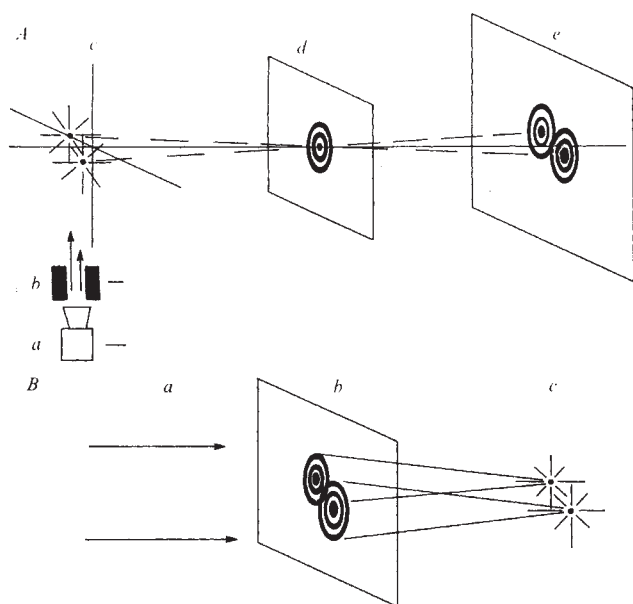


Fig. 1 *A*, Apparatus used to form a shadow of the scatterers: *a*, X-ray generator; *b*, collimator; *c*, scatterers; *d*, Fresnel zone plate; *e*, recording medium. *B*, Formation of an image of the original scatterer: *a*, beam of coherent light; *b*, reduced hologram; *c*, image of point scatterers.

point scatterer is placed at some position on one side of the zone plate. The scattered radiation field casts a shadow of the zone plate on a recording medium such as a sheet of X-ray film. The location of the shadow depends linearly on the position of the scattering point and on the distances between zone plate and film. The shadow, copied at a suitable reduction in size, will be a small Fresnel lens capable of focusing (by diffraction) a beam of coherent light to a point image of the original scatterer (Fig. 1*B*). The focal point of the Fresnel lens is dependent on the size of the shadow and thus on the distance of the scatterer from the aperture. For an actual extended object each point in the object will act as a point scatterer, producing its own Fresnel lens shadow and each lens will reproduce its

associated point scatterer independently of all of the others. The superposition of the point images will yield an image of the extended scattering object, and because each plane of the object is at a different distance from the zone plate, it will focus at a different distance from the shadow transparency. As a result, the image will have three-dimensional information which can be viewed tomographically (plane by plane).

The Fresnel zone pattern is familiar as the hologram of a point, and the optical hologram of an extended object can be thought of as the summation of many point holograms (Fresnel zones) formed by each point of the object. This analogy with optical holography implies that the complex shadows of the Fresnel zone aperture can be thought of as a pseudo-hologram of the scattering object, formed by shadowing rather than interference. Decoding can be thought of as the reconstruction of the actual scattered wavefronts.

The imaging system is illustrated in Fig. 2*A*. The primary source was a standard industrial X-ray generator with the beam collimated to a 3-cm circular cross-section and located about 30 cm from the scatterer. For most of the exposures the generator was run at a potential of 90 kV and a beam current of 5 mA for an average exposure time of 45 s. The zone plate had 30 zones with a maximum diameter of 10 cm and a minimum width of 0.86 mm. It was used in the on-axis mode and positioned about 8 cm from both the centre of the scatterer and the film in such a way as to view the 90° scattered component. This configuration



Fig. 3 The scattering object.

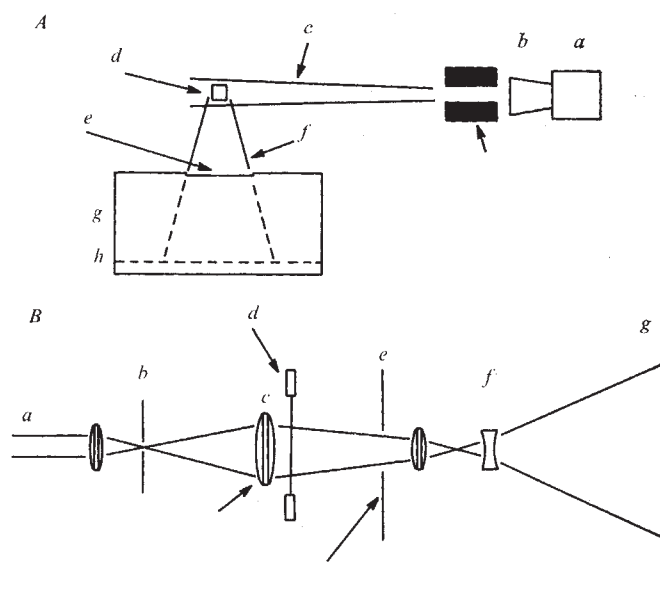


Fig. 2 *A*, Imaging system: *a*, X-ray generator; *b*, collimator; *c*, primary field; *d*, scattering object; *e*, zone plate; *f*, scattered field; *g*, shielding; *h*, X-ray cassette. *B*, Optical reconstruction system: *a*, laser beam; *b*, spatial filter; *c*, transform lens; *d*, hologram; *e*, iris and shutter; *f*, telescope; *g*, image plane.

of the imaging system has theoretical resolution of approximately 2.0 mm as determined by the relative position of the zone plate and the width of the outermost transparent ring.

The opaque rings were composed of a lead powder and glue matrix with an aluminium backing plate comprising the transparent rings. The recording system consisted of an X-ray film cassette with high speed intensifying screens and fast film. The resulting transparencies were copied at a reduction of approximately 64:1 and placed in the optical reconstruction system (Fig. 2*B*). Light from the helium-neon laser was passed through a spatial filter to clean up the beam and then reconverged through the pseudo-hologram. The real image was initially very small and was therefore magnified and focused on a high speed photographic film packet on which it was recorded.

The scattering object is shown in Fig. 3. The letters were made of aluminium, each approximately 25 mm high, 18 mm wide and 6 mm thick. The aluminium support rod was 2.5 mm in diameter and the letters were positioned with lateral displacement 6 mm and depth separation 12 mm. The purpose of the depth separation was to demonstrate the

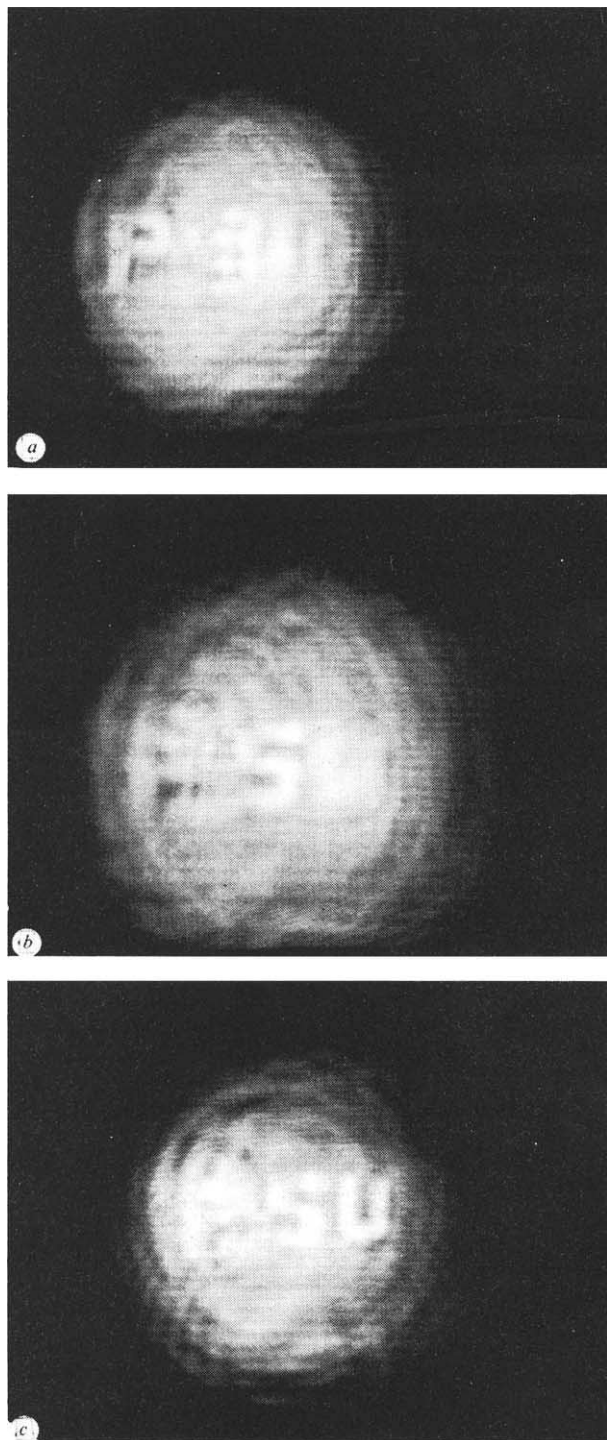


Fig. 4 Reconstructed images of the scattering object (Fig. 3) showing the three-dimensional capabilities of this type of imaging: *a*, focused in the 'P' plane; *b*, focused in the 'S' plane; *c*, focused in the 'U' plane.

three-dimensional capabilities of this method of imaging and its effects can clearly be seen in the reconstruction.

Figures 4*a*, *b*, and *c* show the reconstructed images. The tomographic effect is clearly demonstrated, with each reconstruction corresponding to a different image plane. The theoretical resolution of the system was calculated to be approximately 2.0 mm. This is clearly achieved as the dimensions of the centre of the 'P' are 4 mm by 6 mm. The background appearing in the reconstruction is caused by the undiffracted light passing through the pseudoholograms. Removal of the background will result in

higher quality images and can best be accomplished through the use of the zone plate in the off-axis rather than the on-axis mode. In the off-axis mode the undiffracted component and the image are laterally separated.

The results of this work clearly demonstrate that the use of coded apertures permits imaging with scattered radiation of a very different character from visible light. Although this particular work was done using scattered X rays, it is important that the technique is by no means restricted to that type of radiation. Indeed, similar results have been achieved in our laboratory with thermal neutrons scattering from homogeneous material. The use of penetrating radiation is particularly interesting because most of the photons which carry information scatter from the interior of the object. This allows those details of the object which are hidden from photons of visible wavelengths to be viewed. Such a capability could prove very useful in both medical and industrial radiography.

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BIOLOGICAL SCIENCES

Tight binding of RNA polymerase to rDNA genes in *E. coli*

THE synthesis of ribosomal RNA (rRNA) in exponentially growing bacteria accounts for approximately half of the total RNA synthesis, although the ribosomal DNA (rDNA) genes make up only 0.3–0.4% of the genome¹. Electron-microscopic observations² and biochemical data^{3,4} suggest that the frequency of transcription initiation on these genes is much higher than on any other part of the bacterial chromosome. If the same enzyme is responsible for the transcription of all kinds of bacterial RNA, this difference in the initiation frequency might be due either to a specific regulatory factor or to the structure of the relevant interacting macromolecules themselves. If the latter were true, the DNA promoter sequence responsible for the initiation of transcription on rRNA genes should be more active than other promoters. This hypothesis is supported by the

discovery of preferential *in vitro* rRNA synthesis with purified RNA polymerase in the absence of any external factor⁵⁻⁷.

Several attempts have been made to characterise the interaction of RNA polymerase with the DNA promoters. Conditions could be found under which RNA polymerase was bound only on distinct sites of the DNA of several simple DNA phages^{8,9} and these sites seemed to be the promoters¹⁰. Fragmentation of DNA did not disturb the specificity of binding¹¹. Kinetic analysis of dissociation observed at various ionic strengths revealed that the stabilities of these specific enzyme-DNA complexes were different⁸.

The filter binding technique of Jones and Berg¹² as modified by Hinkle and Chamberlin⁸ offered the possibility for us to study the interaction of RNA polymerase with fragments of the much more complex bacterial DNA. The easy and extremely sensitive technique of RNA-DNA hybridisation allowed us to differentiate between rDNA and non-rDNA fragments. We reasoned that if the rDNA

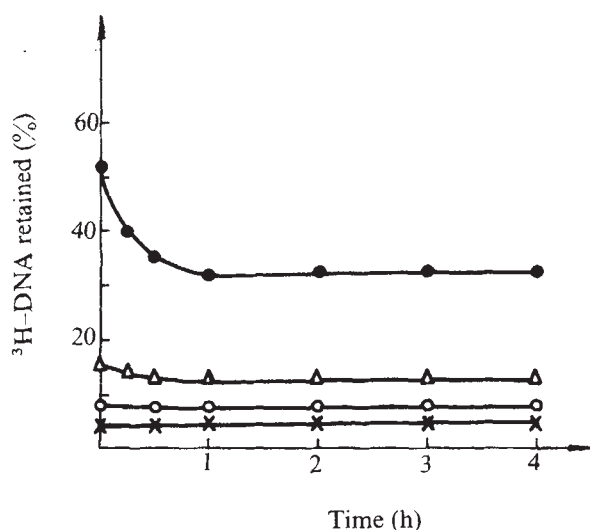


Fig. 1 Highly stable complex of RNA polymerase holoenzyme and bacterial DNA. ³H-labelled DNA from *E. coli* MRE 600 (specific activity 14,200 c.p.m. μg⁻¹) was sheared by passing it several times through an injection needle (No. 16) with syringe to get DNA fragments of average double stranded molecular weight 12×10^6 as determined by gradient centrifugation in neutral sucrose density gradient¹⁷. ³²P-labelled λ phage DNA was applied as molecular weight standard. In order to remove single-stranded ends from the DNA fragments 50 μg sheared DNA was incubated for 5 min at 37° C in 200 μl of reaction mixture containing 0.015 M sodium acetate pH 4.6, 0.05 M NaCl, 1 mM ZnSO₄, 5% glycerol and 5 μg nuclease S₁ prepared from *Aspergillus oryzae* crude alpha-amylase¹⁸. The reaction was stopped by the addition of 10 μl M Tris HCl pH 8.4, to bring the pH to 7.0. RNA polymerase holoenzyme was prepared from *E. coli* MRE 600 according to Burgess¹⁹ as modified by Nüsslein *et al.*²⁰. The enzyme synthesised 8–10% rRNA on total *E. coli* DNA template. 4.5 μg nuclease S₁-treated ³H-DNA was preincubated for 15 min at 37° C in 0.8 ml binding buffer⁸. Binding reaction was started by the addition of RNA polymerase. After an additional 20 min incubation, 45 μg unlabelled T₇ phage DNA was added to dilute out the dissociated enzyme molecules. During subsequent incubation 100 μl aliquots were removed at the times indicated, diluted with binding buffer to 1 ml, filtered immediately through Millipore HAMP 02500 (0.45 μm) filters under 2 cm of mercury vacuum, washed with 3 ml of binding buffer, dried and counted in a toluene base scintillation fluid. A blank (0.2–0.3%) representing the amount of ³H-labelled DNA retained by the filter in the absence of RNA polymerase were subtracted from all points. The amounts of RNA polymerase holoenzyme added were 1 μg ●; 0.35 μg Δ; 0.15 μg ○; 0.1 μg ×.

promoters are indeed more active, then, under certain conditions which favour the dissociation of less stable complexes, that is, at raised ionic strengths, the proportion of rDNA should increase in the bound fraction, provided the fragments are of appropriate size.

When ³H-labelled *Escherichia coli* DNA was mixed with highly purified RNA polymerase holoenzyme in a proportion of 4.5:1 by weight, approximately 50% of the DNA was retained on the nitrocellulose filters as DNA-protein complex. On dilution with unlabelled homologous or heterologous T₇ phage DNA the filter-bound radioactivity rapidly dropped to 30%, but the dissociation did not go further (Fig. 1). The non-dissociated complex corresponds to the highly stable complex defined by Hinkle and Chamberlin⁸. At a lower polymerase:DNA ratio practically all the enzyme molecules could be found in such a highly stable complex. The proportion of rDNA in this highly stable complex was approximately the same as in the total DNA. If, however, the ionic strength of the medium was increased, the complex progressively dissociated (Fig. 2) and the amount of DNA retained on the filters decreased. The rDNA content of the filter-bound DNA was inversely proportional to the total amount of DNA bound (Table 1). In order to prove that in DNA-RNA polymerase complexes observed at different ionic strengths the enzyme was bound to specific regions of DNA (that is, to promoters) dissociation experiments were repeated in the presence of heparin which inactivates free and non promoter-bound RNA polymerase^{13,14}. Heparin destroyed a fraction of enzyme-DNA complexes and the rDNA enrichment in the heparin and salt-resistant complexes was more pronounced (Table 1). Our results do not prove that the observed stability is an exclusive property of rDNA promoters. But in view of the magnitude of rDNA enrichment and the average size of fragments it is obvious that these promoters belong

Table 1 Tight binding of RNA polymerase to rDNA

Salt-resistant fraction M (NH ₄) ₂ SO ₄	Without heparin		With heparin	
	³ H-DNA retained (c.p.m.)	rDNA content (%)	³ H-DNA retained (c.p.m.)	rDNA content (%)
0.0 (control)	7,030	0.33	4,940	0.41
0.125	3,630	0.79	2,630	1.20
0.250	1,350	1.87	1,030	2.41
0.375	590	4.26	490	4.95
0.500	370	8.45	310	10.60

14 μg ³H-DNA (average double-stranded molecular weight 12×10^6) treated with nuclease S₁ as described in Fig. 1 was preincubated in 0.8 ml binding buffer for 15 min at 37° C. Binding reaction was started with the addition of 0.6 μg RNA polymerase holoenzyme. After 20 min binding buffer or heparin (1 mg ml⁻¹) was added and 10 min later dissociation was initiated with ammonium sulphate. To measure the amount of salt-resistant enzyme-DNA complex 100 μl aliquots were diluted after 60 min incubation with binding buffer to 1 ml and filtered. For the estimation of rDNA content the rest of the reaction mixture was diluted with 3 ml of binding buffer and filtered. Approximately 95–97% of this filter-bound DNA can be recovered by incubating the filters for 3 h at 37° C in 2 ml of 0.15 M NaCl, 0.015 M Na citrate, pH 7.0 containing 200 μg ml⁻¹ self-digested pronase. The rest of the bound DNA was liberated by soaking the filters in 1 ml of 0.3% sodium dodecylsulphate at 100° C for 20 min. The pronase and SDS fractions were pooled, exhaustively dialysed against 0.01 M NaCl, 0.01 M Tris HCl, pH 7.0. DNA was denatured by alkali and hybridised with ³²P-labelled rRNA, specific activity 1.25.10⁶ c.p.m. μg⁻¹ on Millipore filters²¹. ³²P-labelled rRNA was prepared from *E. coli* MRE 600 growing on 121 pepton medium²² containing 12.5 mCi KH₂ ³²PO₄ per 100 ml of culture. Before collecting the cells a 200-fold excess of potassium phosphate was added for a generation to chase radioactivity from the messenger RNA. Ribosomes were prepared and purified²³. rRNA was prepared by phenol extraction and ethanol precipitation and further purified on methylated albumin-Kieselguhr column with a linear NaCl gradient. The hybridisation blank of rRNA prepared in this way was less than 0.02% of input radioactivity.

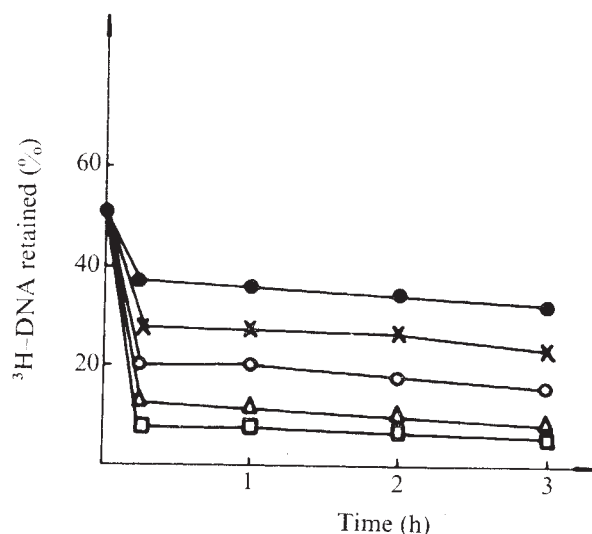


Fig. 2 Effect of ionic strength on dissociation of the highly stable complex. 1 μ g RNA polymerase and 4.5 μ g 3 H-DNA treated with nuclease S_1 was mixed to prepare the enzyme-DNA complex as described in Fig. 1. The complex was dissociated by adding increasing concentrations of ammonium sulphate in the presence of 45 μ g unlabelled T₇ phage DNA. At the times indicated 100 μ l aliquots were removed, diluted with binding buffer to 1 ml and filtered. The final concentrations of ammonium sulphate were 0.05 M $\bullet$; 0.10 M $\times$; 0.15 M $\circ$; 0.20 M $\triangle$; 0.25 M $\square$.

to a small group of promoters, representing only a fraction of the total, which might be characterised by extremely tight binding.

As expected, the rDNA enrichment in the salt-resistant complex was smaller if the DNA fragments were larger (Table 2). Under a molecular weight of about 10^6 however, the enrichment effect could not be enhanced, small fragments bound very poorly and the effect was not reproducible.

Elution of the tightly bound complex from the filters was very difficult. One hundred per cent recovery could be achieved only after prolonged pronase digestion plus hot SDS treatment. If the pronase treatment was shorter, only 95% of the bound DNA could be eluted and this fraction was not enriched with rDNA; the bulk of the rDNA was found in the partly pronase-resistant 5% (Table 3). It is known that RNA polymerase becomes more resistant to proteolytic enzymes after binding to DNA, and this is thought to be due to a conformational change^{15,16}. Our result suggests that the strong binding of the polymerase at the rDNA promoters might effect this conformational change, rendering the complex even less susceptible to pronase attack.

The demonstration of an extremely strong binding of RNA polymerase to DNA fragments carrying the rDNA

Table 3 Distribution of rDNA sequences between pronase sensitive and pronase resistant fractions of enzyme-DNA complex

	rDNA content (%)
Pronase-sensitive fraction	0.19
Pronase-resistant fraction	3.1
Total DNA (control)	0.3

A highly stable complex of RNA polymerase holoenzyme and DNA was prepared as described in Fig. 1. The whole reaction mixture without addition of ammonium sulphate was diluted with 3 ml of binding buffer and filtered. Ninety-two per cent of the filter-bound DNA was recovered after incubation for 60 min at 37°C in 0.15 M NaCl, 0.015 M Na citrate, pH 7.0, containing 200 μ g ml⁻¹ self-digested pronase. The rest of the filter-bound DNA, the pronase-resistant fraction, was liberated by an additional pronase digestion for 2 h and hot SDS treatment. Both pronase-resistant and pronase-sensitive fractions were hybridised with 32 P-labelled rRNA.

genes strongly supports the notion that DNA structure itself might be responsible for the high frequency of transcription on these genes observed both *in vivo* and *in vitro*, although it does not exclude additional regulatory factors.

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Probable linkage of LCAT locus in man to the α haptoglobin locus on chromosome 16

LECITHIN: cholesterol acyltransferase (LCAT) catalyses the esterification of cholesterol in plasma. Familial LCAT deficiency was first described in 1967 (ref. 1); in 1970 a second² family and in 1973 a third, with the same disease, were found in Norway. Seven patients with ages of 26–60 yr

Table 2 Effect of template molecular weight on rDNA enrichment

Salt-resistant fractions M (NH ₄) ₂ SO ₄	rDNA content (%)		
	Molecular weight of DNA		
	34 $\times 10^6$	20 $\times 10^6$	12 $\times 10^6$
0.0 (control)	0.27	0.34	0.32
0.125	0.39	0.50	0.68
0.250	0.61	0.93	1.40
0.375	0.87	1.86	3.43
0.500	1.05	2.15	8.37

Salt-resistant fractions of the RNA polymerase holoenzyme-DNA complex were prepared as described in Fig. 1 applying DNA of different molecular weights. The rDNA content of these fractions was determined by hybridisation with 32 P-labelled rRNA.

are now known in that country³.

Clinically the disease is characterised by proteinuria, corneal opacities and normochromic anaemia with decreased erythrocyte life span. The plasma cholesteryl ester and lysolipid concentrations are decreased, and free cholesterol and lecithin usually increased. Studies of the LCAT-deficient patients have increased our knowledge of normal lipoprotein metabolism in man⁴⁻⁷. The fact that the patients develop atherosclerotic lesions when young, even though they have extremely low levels of plasma cholesteryl esters, may help our understanding of the development of atherosclerotic lesions.

The first three families with this disorder have been traced back five to eight generations and most members have come from the same area^{8,9}. No inbreeding occurred between the maternal and paternal branches of the families. Moreover, no direct connections have been found between the families. The familial occurrence, the horizontal distribution within families and the fact that the disease manifests itself in both sexes make an autosomal, recessive inheritance pattern likely. The presumed heterozygous carriers (parents and children of the patients) are clinically and biochemically normal. One possibility is that the genetic basis of this disease is the presence of an LCAT-deficiency gene in double dose. This defective gene, operating in all three Norwegian families, is probably the result of one mutational event which occurred at least 250–300 yr ago.

In an attempt to characterise further the genetic defect operating in LCAT deficiency we carried out linkage studies to genetic marker systems currently in common use. The following markers were examined in the three families: A₁A₂BO, Rh (CDEce) MNS, Kell, Fy(b), haptoglobin (Hp), group specific component (Gc), transferrin (Tf) third component of complement (C3), phosphoglucomutase (PGM₁), erythrocyte acid phosphatase (EAP), alanine aminotransferase (GPT) and HL-A.

The studies excluded close linkage between the LCAT gene and the genes determining the marker systems Rh, Duffy, Gc,

C3, PGM₁, EAP and HL-A. With respect to the other markers, these families were not sufficiently informative with one exception: nonrandom assortment was observed between LCAT deficiency and Hp types (Fig. 1). The appearance and inheritance pattern of the Hp phenotypes were quite regular in all family members studied, including 15 more distant relatives. When tests for linkage were carried out using a computer program (MOSM) described by Gedde-Dahl *et al.*¹⁰, a combined lod score of 2.81 at a recombination fraction of 0.00 was found. At a recombination fraction of 0.05 the lod score is 2.48. This is very close to a lod score of 3.00 which is usually regarded as good evidence for chromosomal linkage. Given close linkage no recombinants can be counted between the α haptoglobin locus and the LCAT locus.

The total posterior probability of linkage calculated according to Smith¹¹ was 0.81. A linkage disequilibrium exists, with five out of the six parents handing over the LCAT deficiency gene to their children together with the Hp 1 type. The probability of such a constellation appearing by chance is less than 0.04. Through the period of at least six to eight generations that this gene has existed, only one proven crossover event has taken place. This speaks in favour of close linkage, with a probable recombination fraction of less than 0.05 between the two loci.

Robson *et al.*¹² have shown that the α haptoglobin locus, which determines the genetic variability of haptoglobin, is situated between the middle and terminal point of the long arm of chromosome 16. No case of linkage to this locus has been reported so far.

Our work indicates rather strongly that the LCAT locus is situated on chromosome 16, close to the α haptoglobin locus. This is of interest to geneticists as a new linkage group is added to the human chromosome map. Prenatal diagnosis are not possible at present because haptoglobin is not formed in significant quantities in the foetus.

Our results suggest that the basis of this disease, which reveals an inborn error of lipid metabolism, is a single genetic defect causing a lack of LCAT activity in plasma.

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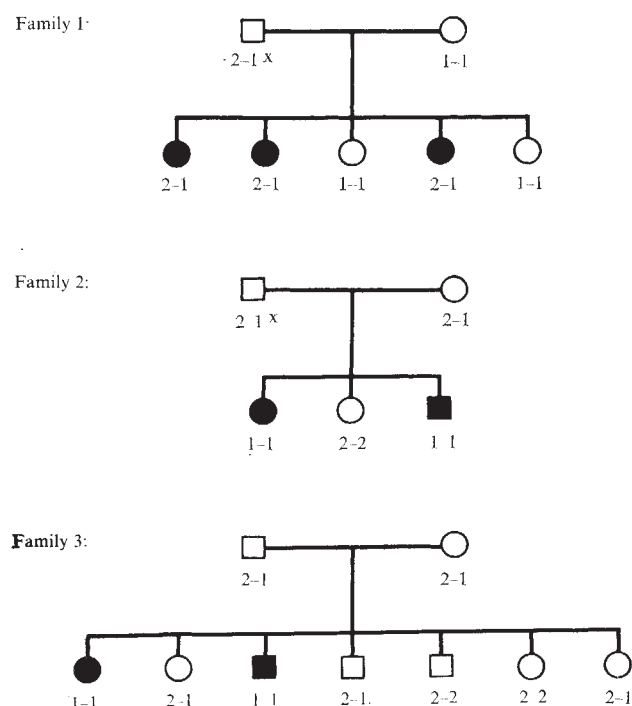


Fig. 1 Hp types in the members of the three families in which LCAT deficiency has been found. x, Hp types deduced from the types of the other family members; ●○, females; ■□, males. ■, ●, Patients with LCAT deficiency; □, ○, family members with no signs of disease.

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Photoreactivation of sister chromatid exchanges induced by ultraviolet irradiation

In contrast to the inefficiency of ionising radiations in the induction of sister chromatid exchanges (SCEs)^{1,2}, ultraviolet light can cause a striking increase in the SCE frequency in mammalian chromosomes^{3,4}. It has been shown in Chinese hamster cells that the SCE formation requires postirradiation DNA synthesis, and that a single ultraviolet irradiation can induce SCE repeatedly for several cell cycles⁴. As pyrimidine dimers are the major DNA lesions caused by ultraviolet light and as Chinese hamster cells have a poor ability to excise them⁵, the delayed formation of SCE seems to be due to unexcised pyrimidine dimers. This possibility was tested here with the use of a rat kangaroo cell line possessing a photoreactivating enzyme⁶⁻⁸ to see if the frequency of ultraviolet-induced SCE was actually reduced when cells were post-treated with visible light.

The female rat kangaroo cell line, Pt-kl, had a doubling time of about 38 h in our culture conditions⁴. In the first experiment, exponentially growing cells were labelled with ³H-thymidine (0.2 μ Ci ml⁻¹, specific activity 12.1 Ci mmol⁻¹) for 8 h, washed twice with fresh medium containing 10 μ M cold thymidine, drained, and then exposed to ultraviolet light at doses ranging from 20–80 erg mm⁻² at an incident dose rate of 5 erg mm⁻² s⁻¹. The cultures were then incubated in normal medium containing cold thymidine. One set of duplicate cultures was kept in the dark, while the other was illuminated for 12 h with visible light from a 20-W fluorescent 'blue light' bulb (National) located 10 cm above the culture dishes. After exposure to visible light, the cultures were returned to the dark. The cells were collected 68 h after ultraviolet irradiation. Colcemid (0.05 μ g ml⁻¹) was added to culture medium 8 h before collection, chromosome preparations being made according to the conventional air-drying method. At cultivation, most labelled cells were in the second postlabelling mitosis. Slides were coated with emulsion (Sakura, NR-M2) and, after appropriate exposure times, were developed and stained with Giemsa. The frequency of SCE in the largest (No. 1) chromosomes is plotted against ultraviolet dose in Fig. 1. Ultraviolet irradiation at 80 erg mm⁻² caused about a four-fold increase in the SCE frequency above a control level, whereas illumination of ultraviolet irradiated cells with visible light lowered the SCE frequency markedly. Thus, at 80 erg mm⁻² of ultraviolet light, it was only twice the control level. The only ultraviolet-induced DNA lesion that is known to be reversible by photoreactivation is pyrimidine dimer⁹. Now that most SCEs induced by ultraviolet light are photoreactivable, it may be safely concluded that the lesions leading to the formation of SCEs are pyrimidine dimers.

This conclusion was confirmed by experiments in which ultraviolet irradiated cells were kept in the dark for various times before being submitted to photoreactivation treatment and ³H-labelling. The results are shown in Fig. 2. When cultures were kept in the dark, the SCE frequency decreased gradually with time, but it was significantly higher than the control level even 164 h after irradiation. By that time the cells might have undergone division at least three or four times, as mitotic delay caused by the ultraviolet dose used was found to be less than 24 h during this culture period.

These results indicate that a substantial number of pyrimidine dimers remains unexcised, even though the rat kangaroo cells are reported to carry out repair replication after ultraviolet exposure⁷, and that the cells might have a repair mechanism that renders them tolerant to the presence of unexcised pyrimidine dimers. These dimers, if not repaired by photoreactivation, would repeatedly provoke SCE before they are either removed by the excision mechanism and/or diluted through consecutive

cell divisions. Previous work has shown that induction of SCE by ultraviolet light requires a postirradiation replication and is inhibited by caffeine post-treatment¹. The involvement of pyrimidine dimers in this phenomenon was confirmed in our study. These features seem to indicate that SCE is closely related to postreplication repair of ultraviolet-induced DNA damage¹⁰⁻¹³.

The exact mechanism underlying the induction of SCEs is still unknown. At least, it is probable that the formation of SCEs involves double-strand exchange: assuming that each chromatid contains a single DNA duplex^{14,15}, only a parental strand in one of the sister chromatids is assumed to contain ³H. A switch of silver grains on an autoradiogram might therefore be an outcome of exchange between the labelled parental strand in one chromatid and an unlabelled daughter strand in another. To achieve this step while avoiding an unwinding difficulty, simultaneous exchange between the remaining two strands of the same polarity in the two sister chromatids would be required. Considering this aspect, Whitehouse's model¹⁶, which was originally proposed to explain genetic crossing over, also seems to be a feasible explanation for formation of SCEs. According to his model, the initial step of crossing-over is the formation of staggered breaks of two nascent, complementary strands from two DNA molecules, which is then followed by a limited replication of the broken ends along the parental template strands. In the induction of SCEs by ultraviolet

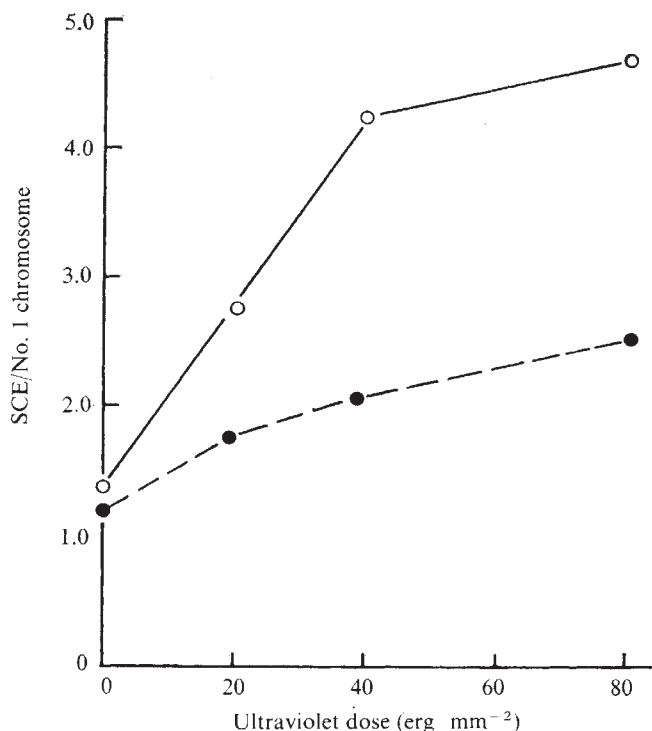


Fig. 1 Dose response curves of (SCEs) with or without photoreactivation treatment. SCE was scored only in No. 1 chromosomes in cells at a diploid level which constituted about 60% of the cell population. Any unlabelled region following a labelled segment of a chromatid and matched by the opposite pattern in its sister chromatid was scored as one exchange. The presence of an unlabelled region within a labelled segment together with the presence of at least three grains on the sister chromatid opposite the region was regarded as evidence that two exchanges had occurred. In ultraviolet irradiated cultures, a considerable number of cells had aberrant chromosomes, so that only intact chromosomes were selected for scoring. No account was taken of whether other chromosomes within the cell were aberrant or not. Isolabellings were omitted from the score. ○, Ultraviolet only; ●, ultraviolet and visible light.

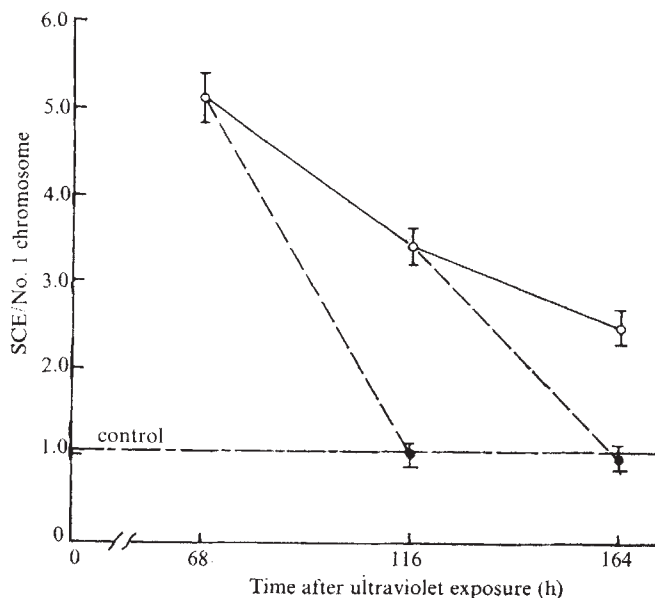


Fig. 2 Delayed formation of SCEs after a single ultraviolet exposure at a dose of 60 erg mm^{-2} . The exchange frequency was determined for only No. 1 chromosomes in the second postlabelling metaphase cells. One set of cultures was labelled with ^3H -thymidine for 8 h and exposed to ultraviolet light at 60 erg mm^{-2} . It was incubated in the dark and fixed for chromosome preparation at 68 h after irradiation. Other sets of cultures were first exposed to ultraviolet light at 60 erg mm^{-2} and incubated in the dark for times before being submitted to photoreactivation treatment and ^3H labelling: one of duplicate cultures was for 12 h illuminated with visible light from 28 h to 40 h after ultraviolet irradiation, while the other was kept in the dark. Both were then labelled in the dark for 8 h, incubated further and fixed 68 h later, that is, 116 h after ultraviolet irradiation. A similar procedure was applied to the remaining set of cultures: one of them was illuminated with visible light from 76 h to 88 h after ultraviolet irradiation, while the other was kept in the dark before ^3H labelling. — — —, Visible light; —, ultraviolet light.

light these steps may correspond to the formation of gaps in the two newly synthesised strands at adjacent positions and to the *de novo* DNA synthesis detected previously^{10,11}, although it may still be that the staggered breaks are actually simple nicks made enzymatically, provided that, as claimed by Chiu and Rauth¹⁷, most dimer sites do not involve gap formation after exposure to small amounts of ultraviolet light. The most important part of his model is his proposal that cross-over molecules form by pairing of the two overlapping sectors of the daughter strands of the broken ends. This process effects a crosswise reunion of daughter DNA strands of the two future sister chromatids. In the light of recent findings about the middle repetitive nucleotide sequence in eukaryote DNA¹⁸⁻²⁰, it is tempting to speculate that such a pairing process may be facilitated by short repetitive nucleotide sequences interspersed between unique sequences.

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Molecular basis of control of mitotic cell division in eukaryotes

MITOTIC division in *Physarum polycephalum* is preceded by a marked increase in the phosphate content of very lysine-rich histone (Pp. F1) (ref. 1) due to phosphorylation of serine residues probably at specific points in the amino acid sequence^{2,3}. The phosphate content of F1 histone has been measured by a number of groups under a variety of conditions in an attempt to understand the role of F1 phosphorylation and three facts have been established.

First, F1 phosphate content is positively correlated with cell replication rate in calf⁴, cultured hepatoma cells^{5,6}, normal and regenerating rat liver⁷ and a variety of rat and mouse tumours⁸, giving an important indication that the correlation is universal.

Second, some F1 phosphorylation takes place during S phase. In regenerating rat liver F1 phosphorylation occurs in the few hours before mitosis⁷⁻⁹ but it is not possible to distinguish, in this system, between S phase and G2 phase and this high phosphate content could not be correlated either with DNA synthesis or with initiation of mitosis. Histone phosphorylation has been studied in cultured mammalian cells synchronised at mitosis using a blocking agent and mitotic selection¹⁰ which gives synchrony through G1 phase and into S phase but not in G2 phase¹¹. Pulse labelling experiments have shown increased phosphorylation of F1 in S phase¹⁰. Different experiments using long term double labelling and the precise natural synchrony in *P. polycephalum* have shown that there is no change in the overall phosphate content per molecule of Pp. F1 during S phase¹.

It is important to determine whether these data are consistent with the mammalian pulse labelling experiments and so the *P. polycephalum* data have been transformed to predict the results of a pulse label experiment such as those carried out in mammalian systems. The transformation (Fig. 1) shows that a peak of phosphorylation, as measured by pulse labelling, would appear in S phase in *P. polycephalum*, in agreement with the mammalian observations. The peak is due to histone synthesis, which requires some phosphorylation to occur in order to maintain the constant overall phosphate content per molecule. These results throw considerable doubt on the significance of the S phase phosphorylation although the possibility that different sites of

phosphorylation are involved cannot yet be ruled out. The doubt has been strengthened recently by studies of the coupling of S phase phosphorylation and DNA synthesis¹², and by the work of Marks *et al.*¹³ with synchronous HeLa S-3 cells.

Third, most Pp. F1 phosphorylation occurs in late G2 phase (Fig. 1) and at this time there is also a large increase in overall phosphate content per molecule¹. Increased F1 phosphorylation has also been observed in mammalian cells in late G2 phase¹¹, mostly in the 3 h before metaphase¹⁴, exactly in agreement with the *P. polycephalum* results. Further, an increased F1 phosphate content occurs in mammalian metaphase cells¹⁵ and dephosphorylation occurs when these cells move into G1 (ref. 16). These data are consistent with the very precise timing in *P. polycephalum* which located the peak of maximum Pp. F1 phosphate content at the time of chromosome condensation¹ and this is proposed as the initiation step for mitosis¹⁷. The nuclear F1 histone phosphorylating enzyme activity has been found to peak dramatically at the time during the mitotic cycle when the net rate of phosphorylation of Pp. F1 is a maximum implying that the change in enzyme activity 'triggers' the phosphorylation¹⁷. Further we have shown that there is a remarkable correlation between the observed changes in enzyme activity and the data in the literature concerning the expected behaviour of the mitotic trigger in *P. polycephalum* and it has been proposed that these events are linked¹⁷. As a direct test of the proposal, the effect of the addition heterologous F1 histone phosphokinase on the cycle time of the naturally

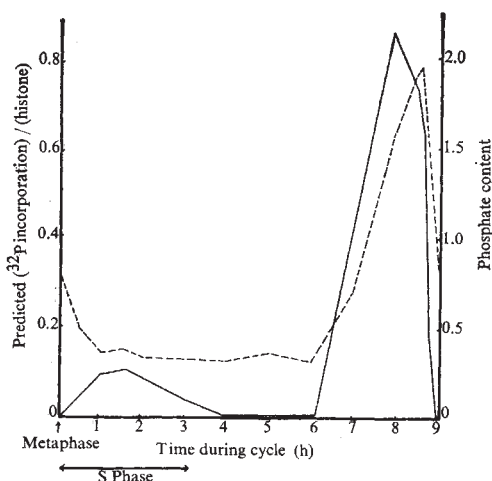


Fig. 1 The pulse length was 1/8 of a generation time in the mammalian experiments¹⁰ which is equivalent to 1 h in the *P. polycephalum* cycle. If the net rate of phosphorylation of F1 is dP/dt then a 1 h pulse would show an incorporation

$$\int_{t-1}^t (dP/dt) dt = P_t - P_{t-1} \text{ where } P_t \text{ is the total phosphate content of F1 at time } t.$$

The *P. polycephalum* experiments measured $P_t/N_t = \chi_t$, say, where N_t = total number of F1 molecules at time t . The incorporation expected during a 1 h pulse ending at time t is

$$P_t - P_{t-1} = N_t \chi_t - N_{t-1} \chi_{t-1} = N_t \partial \chi + \chi_t \partial N - \partial \chi \partial N$$

where $N_{t-1} = N_t - \partial N$ and $\chi_{t-1} = \chi_t - \partial \chi$. Therefore incorporation per F1 molecule,

$$(P_t - P_{t-1})/N_t = (N_t \partial \chi + \chi_t \partial N - \partial \chi \partial N)/N_t = \partial \chi + \chi_t \partial N/N_t, \text{ neglecting } \partial \chi \partial N$$

The first term, $\partial \chi$, represents incorporation due to a change in the overall phosphate content of the F1 and the second term represents net incorporation due to maintaining the overall phosphate content per molecule constant in the face of synthesis of new F1. Any turnover of F1 phosphate would raise the level of the curve but this has been ignored. N_t and ∂N were estimated for *P. polycephalum* by assuming a constant ratio of Pp. F1 histone synthesis to main band nuclear DNA synthesis²⁵ and using published data on DNA synthesis¹⁶. χ_t and $\partial \chi$ were obtained from our previous measurements of Pp F1 phosphate content¹. ———, Calculated phosphorylation (simulated label experiment); — — — measured phosphate content¹.

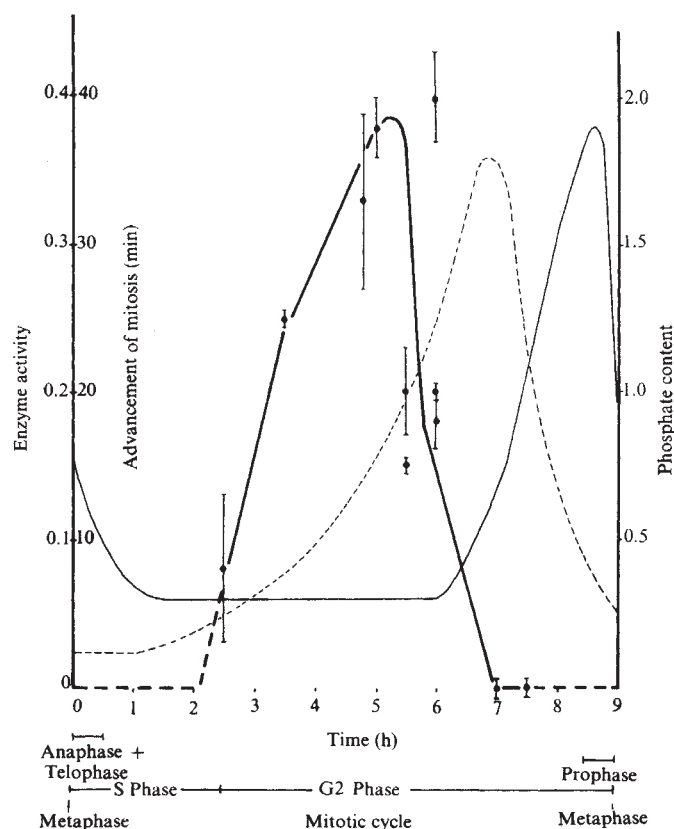


Fig. 2 Synchronous plasmodia of *P. polycephalum* were prepared by fusion of microplasmodia on filter paper and grown at 26° C in the dark²⁷. At a known time between mitosis 2 (the second mitosis after fusion) and mitosis 3, the filter paper was cut into quarters and transferred to Petri dishes. Each culture constituted a complete experiment with its own control, conducted in duplicate. Duplicates of the same plasmodium divided within 5 min of each other. Plasmodia were treated by carefully pipetting on their surface 0.1 ml of solution. Enzyme solutions, containing approximately 1.5 mg ml⁻¹ protein had been dialysed overnight against 0.02 M Tris HCl buffer pH 7.2 (ref. 19). The treated plasmodia were starved for 1.5 h to facilitate absorption¹⁹. Fresh medium was then added and the time of the third mitosis determined by microscopic examination of glycerol/ethanol fixed smears under phase contrast²⁸. Advancement of mitosis by F1 phosphokinase is shown as a function of the time in the cycle when the enzyme was added, (●—●) with the errors calculated from the differences between duplicates as in Table 1. For comparison, the level of endogenous enzyme activity¹⁷ (— — —) and Pp F1 phosphate content (——) are also shown together with the timing of the main events in the *P. polycephalum* mitotic cycle²⁶.

synchronous cultures of *P. polycephalum* has been investigated.

F1 histone phosphokinase was prepared by extraction of Ehrlich ascites cell chromatin with 0.4 M NaCl, followed by ammonium sulphate fractionation of the extract in the presence of 10⁻⁵ M cyclic AMP. The protein precipitating between 17.5 and 35% saturation was collected and dissolved in 0.4 M NaCl, 0.01 M Tris pH 7.5. The enzyme activity is similar to that described in metaphase arrested cells by Lake¹⁸. It phosphorylates F1 *in vitro* mainly in the N and C terminal regions at sites whose phosphorylation is associated with growth. It does not require cyclic AMP. The preparation contains only traces of F1 histone kinases, HK₁ (cyclic AMP dependent) and HK₂ (cyclic AMP independent)².

Heterologous enzyme was used because of its availability and because the general features of the process are almost certainly universal. Work is in progress to obtain and use pure homologous and heterologous enzyme, but this is a

Table 1 Control experiments

Treatment begun, (h before expected MIII)	Segments A	Treatment Segments B	Advancement of A before B (min)	Error due to differences* between duplicates (min $\pm$)
3.5	F1 phosphokinase	Buffer	15	0
3.0	F1 phosphokinase	0.1 x phosphokinase	40	3
5.5	F1 phosphokinase	F1 phosphokinase inactivated by freezing and thawing	25	0
4.2	F1 phosphokinase	F1 phosphokinase inactivated by dialysis and storage	33	6
3.0	F1 phosphokinase	F1 phosphokinase inactivated by dialysis and storage	20	0
3.0	F1 phosphokinase	creatine phosphokinase	18	2
4.0	F1 phosphokinase	albumin	38	2

* This error is obtained as [(difference between mitosis times of A segments)² + (difference between mitosis times of B segments)²]/²

long term project.

For each *in vivo* experiment at least two plasmodia of *P. polycephalum* were used. One was not treated in any way and was used as a control for measurements of the normal cycle time. The second was cut into four approximately equal 90° segments at a known time after its second mitosis. Two segments were treated with a blank solution and two with the enzyme solution. The four segments were starved for 1.5 h and then transferred to fresh growth medium (for experimental details see Fig. 2 and ref. 19). Provided the enzyme was added at the correct time in the cycle the enzyme treated segments entered mitosis precisely together up to 40 min before the blank treated duplicate segments which themselves entered mitosis precisely together. The rate of progression through mitosis itself was not affected. The effect of adding the enzyme at different times during the cycle is shown in Fig. 2. This curve correlates with the time course of F1 phosphorylating activity in *P. polycephalum* nuclei, since at about 2 h before mitosis this activity falls off sharply and adding enzyme is found to have no effect, either because the added enzyme is also inactivated or because the enzyme activity is no longer needed by the nucleus. Moreover, added enzyme is most effective when the endogenous enzyme is at one-third to two-thirds of its maximum level. The ineffectiveness of added enzyme early in the cycle is probably due to its instability or to some other preparation for mitosis, such as DNA synthesis, not being complete. The correlation of time of enzyme addition for maximum effect with the previous measurements of endogenous enzyme activity provides strong support for the contention that the observed effect is due to a specific action of F1 phosphokinase and is not a general growth promotion.

The *in vitro* activity of the actual enzyme preparations used for the experiments of Fig. 2 was assayed directly using calf thymus F1 histone as substrate. The specificity of the enzyme for F1 histone in chromatin was demonstrated by incubating calf thymus chromatin in 5 mM MgCl₂ + 50 mM tris-HCl pH 7.5 with γ -³²P-ATP. In the absence of added enzyme, extensive phosphorylation of nonhistone proteins occurred but no histone phosphorylation was observed. In the presence of added enzyme reduced phosphorylation of nonhistone proteins occurred and a large incorporation of ³²P into F1 histone, but not into other histones, was observed²⁰. The significance of the reduced incorporation into nonhistone proteins is unknown but the enzyme clearly phosphorylates specifically F1 histone, in chromatin. It is just conceivable that the unpurified F1 phosphokinase preparation contained some other substance or combination of substances which produced the effects reported (Fig. 2). This possibility can be ruled out completely only by the use of pure homologous F1 phosphokinase. However, some control experiments have been carried out to check if such a coincidence is likely. The enzyme after dialysis against 0.02 M Tris pH 7.2,

can be specifically inactivated by storage for 2 weeks at 4° C or by freezing and thawing four times. Such inactivated enzyme preparations have no effect on the timing of mitosis. Albumin and creatine phosphokinase are also without effect (Table 1). Positive results have only (and always) been obtained with active F1 phosphokinase preparations. Further progress on these lines must await the availability of pure enzyme.

Taken together with the previously reported data on Pp. F1 phosphate content¹, chromatin condensation (for example ref. 21), F1 phosphorylating activity in *P. polycephalum* nuclei¹⁷ and the mitotic trigger²²⁻²⁴, the results reported here provide direct evidence for our previous proposal that F1 phosphokinase triggers an increase in F1 phosphate content which is the initiation step in mitosis. Acceleration of initiation of mitosis has been shown before, by plasmodial fusion^{22,23}, ultraviolet irradiation²³ and plasmodial extracts¹⁹ but this is the first successful attempt to accelerate the initiation of mitosis by a specific substance, F1 histone phosphokinase. The data already accumulated on the mechanism of this control process mediated by F1 phosphokinase fit into a consistent hypothesis (unpublished), and experiments are in progress to test it further and clarify the details. It is interesting that this process probably applies to cancer as well as normal cells.

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*et al.*⁷ using 1 M NH_4Cl . Protein synthesis was measured as incorporation of ^{14}C -phenylalanine in an *in vitro* system containing ribosomes from either unfertilised eggs or blastulae. Only blastula ribosomes had enough natural mRNA attached to give detectable endogenous synthesis (Table 1, experiment 2). Endogenous incorporation with these ribosomes was inhibited strongly by either of the ribosomal extracts (Table 1, experiment 1). Incorporation with ribosomes from both unfertilised eggs and blastulae was stimulated strongly by addition of poly(U). This stimulation was inhibited by ribosomal extracts from both unfertilised eggs and blastulae.

Table 1 Effect of ribosomal extracts on cell-free protein synthesis directed by endogenous mRNA and poly(U)

		<i>in vitro</i> ¹⁴ C-phenylalanine incorporation (c.p.m. per assay)	
Type of mRNA	Source of extract	Using egg ribosomes	Using blastulae ribosomes
Experiment 1			
Endogenous mRNA	none	—	6,520
„	UF egg ribosomes*	—	2,516
„	blastula ribosomes†	—	2,620
Experiment 2			
Endogenous mRNA	none	0	3,740
„ + poly(U)	none	7,430	21,500
„ + „	UF egg ribosomes‡	48	4,940
„ + „	blastula ribosomes§	200	5,860

Eggs and sperm of *Strongylocentrotus purpuratus* were collected and cultivated¹⁹. Ribosomes and their 1 M NH_4Cl extracts were prepared as described by Metafora *et al.*⁷ except that triethanolamine-HCl (K and K Laboratory, Inc.) was substituted for Tris HCl. The ribosome and extract solutions were stored at -80°C . A pH 5 fraction prepared from rat liver supernatants³ contained aminoacyl activating enzymes, tRNA, amino acids, and soluble enzymes. The *in vitro* assay mixture contained in 250 μl : 0.1 M KCl, 5mM MgCl_2 , 0.05 M triethanolamine-HCl (pH 7.4), 6mM mercaptoethanol, 2.5 mM ATP, 0.2 mM GTP, 4 mM 2-phosphoenol pyruvate, 0.35 μg pyruvate kinase, 0.31 μg ^{14}C -L-3-phenylalanine (5.3 mCi mmol⁻¹), pH 5 fraction from rate liver at optimal concentrations (about 30 μl containing about 0.8 mg protein), 2.6 A_{260} units (defined in Fig. 1) of ribosomes, ribosomal extract, 46 μg *, 42 μg †, 58 μg ‡, and 23 μg §, or a compensating buffer and sometimes 50 μg (poly(U)). The reaction mixture was incubated with vigorous shaking for 60 min at 27°C , stopped by the addition of 3 ml of ice-cold 6% trichloroacetic acid (TCA) containing L-phenylalanine, incubated for 10 min at 90°C , chilled in ice, filtered onto membrane disks, then washed with 6% TCA, dried, and counted in a toluene based scintillant. The assay was linear for 50 to 60 min with poly(U) as the messenger and for 20 min with endogenous blastula messenger. Protein content was estimated by the method of Lowry *et al.*²⁰.

Inhibitor of protein synthesis isolated from ribosomes of unfertilised eggs and embryos of sea urchins

It is not yet clear what mechanisms turn on protein synthesis in the first stages of embryonic development. In the sea urchin two mechanisms are debated: activation of ribosomes^{1,2} and activation of mRNA^{3,4}, possibly through adenylation^{5,6}. An inhibitor of protein synthesis has been isolated from ribosomes of unfertilised eggs of *Paracentrotus lividus*^{7,8} by extraction of ribosomes with high concentrations of salt. Metafora *et al.*⁷ found that this inhibitor is a protein, that it is not a ribonuclease and that it decreases ribosomal binding of a synthetic messenger (poly(U)) and of phenylalanine tRNA. They found that this inhibitor was absent from extracts of ribosomes of *P. lividus* blastulae, suggesting that it is inactivated or lost when the eggs are fertilised.

In view of the importance of such a finding for understanding the restriction of protein synthesis in unfertilised eggs, I have investigated similar inhibitors from eggs and embryos of *Strongylocentrotus purpuratus* and determined the distribution of inhibitor between monoribosomes and polyribosomes of echinoid embryos. While confirming the occurrence of inhibitor in unfertilised eggs, I have found it in embryos as well, particularly in monoribosome fractions.

Ribosomal extracts were tested for inhibitory potential in protein synthesis in a cell-free system using both endogenous mRNA and poly(U). Extracts were prepared from ribosomes of unfertilised eggs and blastulae by the method of Metafora

As the inhibitor could have a regulatory function in development, I have measured its concentration in sea urchin eggs and embryos at various stages. High salt extracts from ribosomal pellets were prepared simultaneously from unfertilised eggs, 32-cell morulae, 500-cell blastulae (B1-500) and early mesenchyme blastulae (B1My-1), by the method of Metafora *et al.*⁷ (Fig. 1). Extracts from ribosomes of all early stages were inhibitory. Extracts from equal quantities of ribosomes were progressively less inhibitory when taken from embryos of increasing age. I found that extracts of blastula ribosomes contained about half as much protein as those from unfertilised egg ribosomes. When relative incorporation was plotted against the amount of protein in each extract, the inhibition by extracts from the various stages was similar (Fig. 2). Since there is little change in the total number of ribosomes from fertilisation until mesenchyme blastula stage (about 25 h at 14°C), I conclude that there is a continuous decrease in the amount of extractable inhibitor per embryo during this time.

The ribosomal extracts from these early stages were resolved into between 9 and 11 protein bands on 6 M urea electrophoretic gels¹⁰. Seven of these bands were common to

extracts of unfertilised eggs, morulae and blastulae, and I do not know which might have inhibitory activity.

Previous studies have shown that the polyribosome: monoribosome ratio increases after fertilisation of sea urchin eggs and increases throughout the blastula stage^{11,12}. To see if there was any correlation between the observed amounts of extractable inhibitor and the polyribosome: monoribosome ratio of these embryos, I prepared monoribosomes and polyribosomes of blastulae by sucrose gradient centrifugation. Embryos were homogenised in 0.2 M salt¹³ instead of the 0.4 M salt of Fig. 1. With the lower concentration of salt fewer nonribosomal ribonucleoprotein particles (RNP particles) cosedimented with the monoribosomes and small polyribosomes. The high speed pellets of these fractions were used to prepare the extracts whose inhibitory capacity is shown in Fig. 3. The points for the unfertilised egg extract from Fig. 1 are included for reference although those ribosomes were isolated using different salt concentrations. To obtain an equivalent amount of inhibition by monoribosome and polyribosome extracts, almost three times as many polyribosomes as monoribosomes were needed. For example, 2.4 A_{260} units (defined in Fig. 1) of monoribosomes or 6.7 A_{260} units of polyribosomes gave 40% inhibition.

To determine whether this inhibitor is absent from non-embryonic tissues, extracts were prepared from the ribosomes of adult rat liver, which are known to contain about 90% polyribosomes¹⁴. The effect of these extracts on *in vitro* synthesis of proteins is given in Fig. 3. I found that ribo-

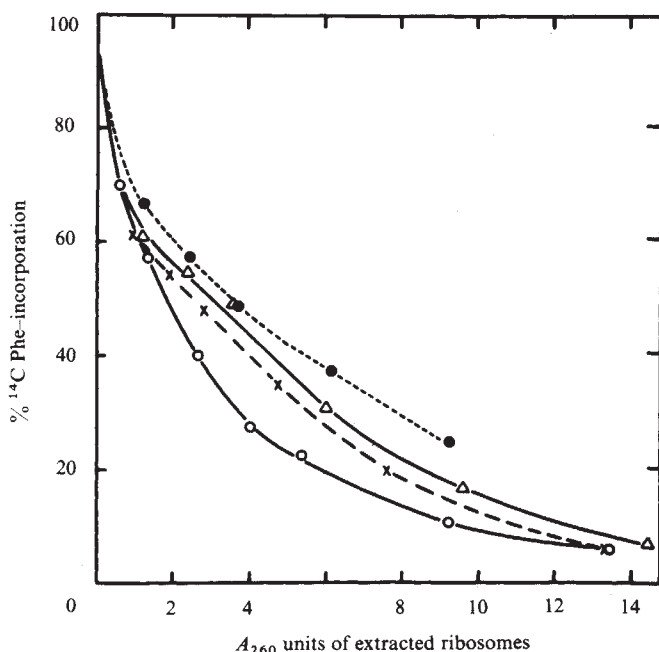


Fig. 1 Quantitative determination of the inhibitory capacity of ribosomal extracts from unfertilised eggs and embryos. The percentage of ^{14}C -phenylalanine incorporation stimulated by poly(U) plus endogenous mRNA is plotted against amounts of ribosomal extracts taken from known quantities of ribosomes. Methods were as described in Table 1 except that for the assays 1.2 A_{260} units of morula ribosomes were added and the incubation time was 45 min. The amount of ribosomal extract varied as noted in the abscissa. The quantity of extract added is measured by the 260 nm absorbance of the ribosomes from which they were taken. This absorbance was measured after the ribosomal pellet had been stirred overnight at 4° C in 1 M NH_4Cl , and after the unsuspended material had been removed by low speed centrifugation. According to convention, 1 A_{260} unit of ribosomes equals the absorption of ribosomes if they were contained in 1 ml and read in a 1 cm cuvette at 260 nm. Ribosomal extracts from unfertilised eggs (○); from 32-cell morulae (×); from 500-cell blastulae (△); and from hatched blastulae at stage B1My-1²¹ (●).

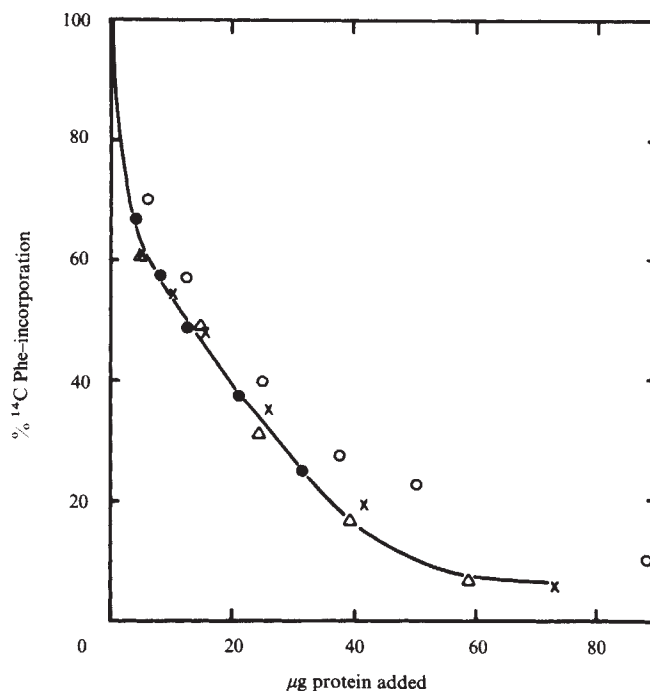


Fig. 2 Inhibition of ^{14}C -phenylalanine incorporation per μg of protein added from ribosomal extracts. Observations and notations are as in Fig. 1. The protein concentration was estimated by a micro Biuret method²².

somes from adult rat liver contained little extractable inhibitor, about 0.1 that of blastulae ribosomes. In summary, Fig. 3 shows that high salt extracts of blastula monoribosomes contain substantially more extractable inhibitor than those of polyribosomes, implying that *in situ* the monoribosomes are inhibited, and that the inhibitor is more abundant in eggs and embryos than in adult tissue.

Preliminary results with RNP particle-enriched fractions do not rule out the possibility that RNP particles which cosediment with monoribosomes also contain inhibitory proteins. The presence of an inhibitory protein on pre-formed messages in such particles could prevent their attachment to ribosomes or their translation once attached.

Ribosomal extracts were tested for possible RNase activity by Ball's method¹⁵. Extracts from unfertilised eggs and blastulae containing 20–40 μg of protein, sufficient to inhibit amino acid incorporation by 60–85%, had less than 1×10^{-10} g RNase. Extracts of rat liver ribosomes sufficient to inhibit amino acid incorporation by 15% also contain negligible amounts of RNase. The rat liver supernatant fraction used in the assays contributed about 2.5×10^{-10} g of RNase. It is unlikely that any observed inhibition is due to RNase activity, for the amount of RNase activity did not correlate with the percentage of inhibition.

The inhibitor is not a GTPase since increasing the amounts of GTP two- and six-fold did not alter the amount of inhibition.

What is the normal function of this inhibitor? While these results and those Metafora *et al.*⁷ and Gambino *et al.*⁸ agree in demonstrating the existence of the inhibitor, these authors indicate that the inhibitor disappears after fertilisation, thereby triggering activation of protein synthesis. In contrast, I have found a gradual diminution in the amount of inhibitor. Therefore, I suggest that this slow loss of inhibitor is responsible for the continuous increase of rate of incorporation of labelled amino acids seen until mesenchyme blastula or early gastrula^{16–18}. The inhibitor seems to be acting at the monoribosome level, so that a

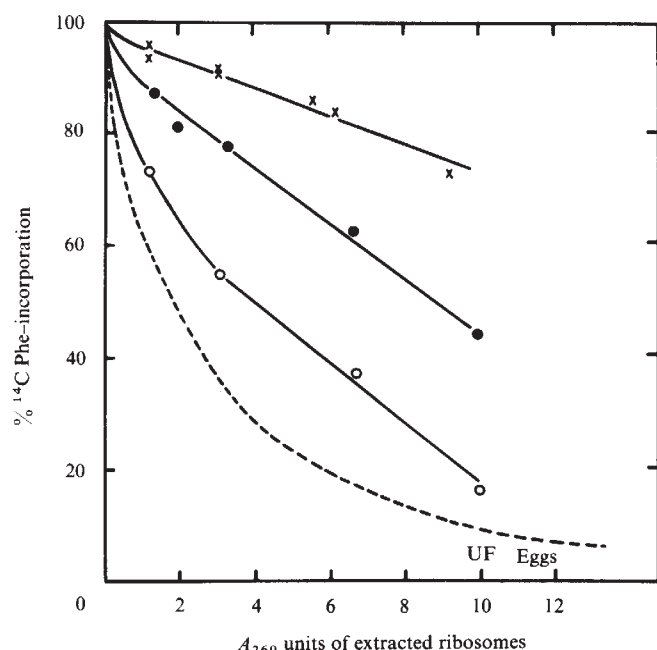


Fig. 3 Inhibitory potency of extracts from different ribosomal fractions, and from adult rat liver. Embryos were slightly older than any described in Fig. 1, being stage B1My-2/Ga-1²¹. Homogenisation and sucrose gradient preparation of mono-ribosomes and polyribosomes were described by Hogan and Gross¹³. Rat liver ribosomal pellets were prepared as described by Stavy and Gross³. Extracts were prepared as described in Table 1. Assay conditions and quantification were as described in Fig. 1. Ribosomal extracts from monoribosomes (○); from polyribosomes containing from 4 to 25 ribosomes per polysome (●); and from rat liver ribosomes (×); unfertilised egg extract of Fig. 1 (---).

gradual loss of inhibitor with time would also account for the continuous increase in the polysome:monoribosome ratio until the mid-blastula stage^{11,12}.

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Stability of the nucleoside composition of tRNA during biological ageing of mice and mosquitoes

In several organisms, alterations in tRNA have been reported to occur during physiological changes. Both qualitative and quantitative changes have been demonstrated in isoaccepting species of various tRNAs¹⁻⁴. Chromatographic changes have often been attributed to changes in nucleoside composition. In *Drosophila*, changes in the chromatographic patterns of certain tRNA species at different developmental stages have been correlated with an enzymatic alteration producing a minor nucleoside, Q⁴. Such differences could be involved in control of protein synthesis at the translational level directly by tRNA species, and (or) in regulation of protein activity by interaction of tRNA with a previously formed protein^{5,6}.

During an investigation of the role of translational macromolecules in biological ageing, column chromatography of aminoacyl-tRNAs from 'mature' and 'old' adults of *Aedes aegypti* revealed variations in patterns of certain isoaccepting tRNAs⁷. A similar study on aminoacyl-tRNA from ageing mice revealed no chromatographic changes⁸. In the liver of ageing rodents, including mice, however, glycine N-methyltransferase activity was reported to increase⁹. It was

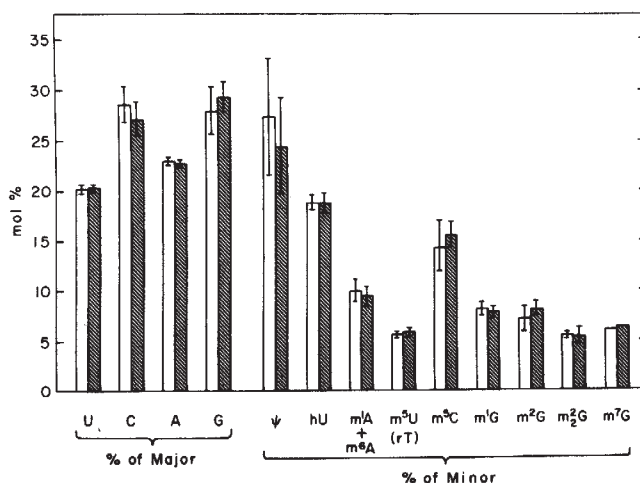


Fig. 1 Nucleoside composition of tRNA from mature and old mosquitoes. Nucleoside compositions were determined by the chemical labelling technique as described in the text. Values plotted are mean $\pm$ s.d. for four determinations on each age with the exception of m⁷G, for which only two analyses were done on mosquito tRNA. Minor/major ratios: mature, 0.109 $\pm$ 0.005; old, 0.106 $\pm$ 0.004. Open columns, mature (10-d-old); hatched columns, old (30-d-old) adult female *Aedes aegypti*.

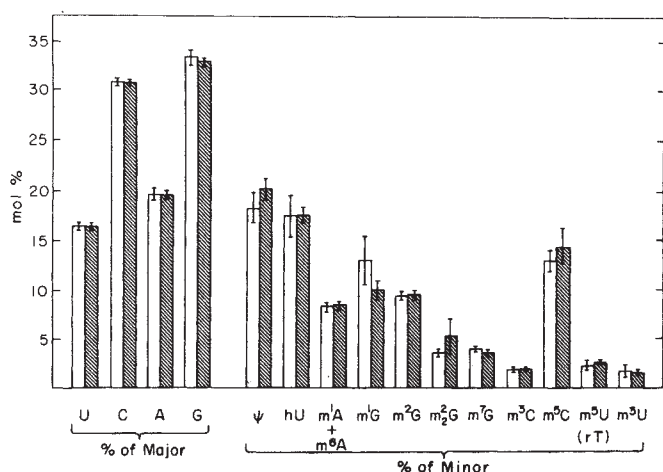


Fig. 2 Nucleoside composition of tRNA from mature and old mice. Determined as in Fig. 1. Minor/major ratios: mature, 0.190 ± 0.013 ; old, 0.193 ± 0.010 . Open columns, mature (12 months old); hatched columns, old (30 months old) female C57BL/6J mice.

suggested that this could lead to undermethylation of tRNA in ageing animals by depletion of S-adenosylmethionine, and by production of S-adenosylhomocysteine which inhibits tRNA methyltransferases¹⁰.

With these observations as background, we have investigated the nucleoside composition of tRNA from mature and old C57BL/6J mice and *Aedes aegypti* to determine if there is any change in this parameter as mature animals age. On the basis of survivorship curves, we have defined mature mosquitoes as 10-d-old adults, and mature mice as 12 months old. Old animals are from the 50% survivorship level, being 30 d for adult mosquitoes and 30 months for mice.

Samples of tRNA were prepared from whole mosquitoes and from mouse liver by phenol extraction, DEAE-cellulose chromatography, and Sephadex G-100 gel filtration. Purity of the preparations was assessed by electrophoresis on polyacrylamide gels and subsequent ultraviolet scanning. All preparations were at least 90% tRNA, the principal contaminant being 5S rRNA.

Nucleoside composition analyses were carried out by chemical tritium labelling exactly as described by the Randerath (ref. 11 and references therein). We were able to quantitate 13 components in mosquito tRNA and 15 in mouse liver tRNA. Inosine seemed to be present in both organisms, but on our chromatograms it was overlapped by the guanosine triol spot and is included with guanosine. A portion of 1-methyladenosine is converted to N⁶-methyladenosine during the analysis, and so these two components were combined in the calculation of composition.

Inspection of fluorograms showed no discernible qualitative differences in nucleoside composition during ageing, that is the same nucleosides were present in tRNA from mature and old animals (data not shown). Figures 1 and 2 show that there are also no significant age-related quantitative changes in tRNA nucleoside composition nor in degree of tRNA modification in the two animal models studied.

It seems remarkable that, at a time when the population has declined physiologically to the point of highest mortality rate, the complex enzyme systems necessary for tRNA maturation are still producing a fully modified product. This indicates that the nucleoside composition of tRNA is an important and tightly regulated parameter for a given organism, or organ therein.

Our results argue against tRNA modifying enzymes having a role in biological ageing. They also point up the danger of interpreting chromatographic changes in tRNA or

changes in activity of tRNA methylases in terms of the final degree of modification without actually analysing the tRNA.

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Relationship between tritiated poly-L-lysine binding and template activity of human chromosomes

It has been reported that cytological preparations of chromosomes can serve as templates in RNA polymerase reactions^{1,2}. The newly synthesised RNA on chromosomes was readily digested with RNase in contrast to RNA which was synthesised in a similar manner but using as a template denatured DNA immobilised on nitrocellulose filters¹. When cytological preparations of chromosomes were pre-treated with poly-L-lysine (PL), no synthesis of RNA could be demonstrated². The implication was that bound PL blocked those sites on DNA template which were responsible for transcription.

We used human metaphase spreads prepared by a standard technique³ as templates for RNA synthesis and for binding tritiated poly-L-lysine (³H-PL). We wished to establish whether both of these processes occur on the same or on different segments of chromosomes. Typical karyotypes of metaphase chromosomes with bound ³H-PL or bound ³H-RNA are shown in Fig. 1. The results of the labelling are summarised in Table 1. In both sets of experiments over 98% of total grains was located over the chromosomes and the remainder over the background. Analysis of a large number of karyotypes (over 100 in total) indicated that the labelling of chromosomes was reproducible (Table 1). Those sites on A chromosomes that have highest template activity also show a high affinity for ³H-PL. Although structural organisation of chromosomes necessary for both functions is not known in detail⁴, it is likely that relatively exposed segments of

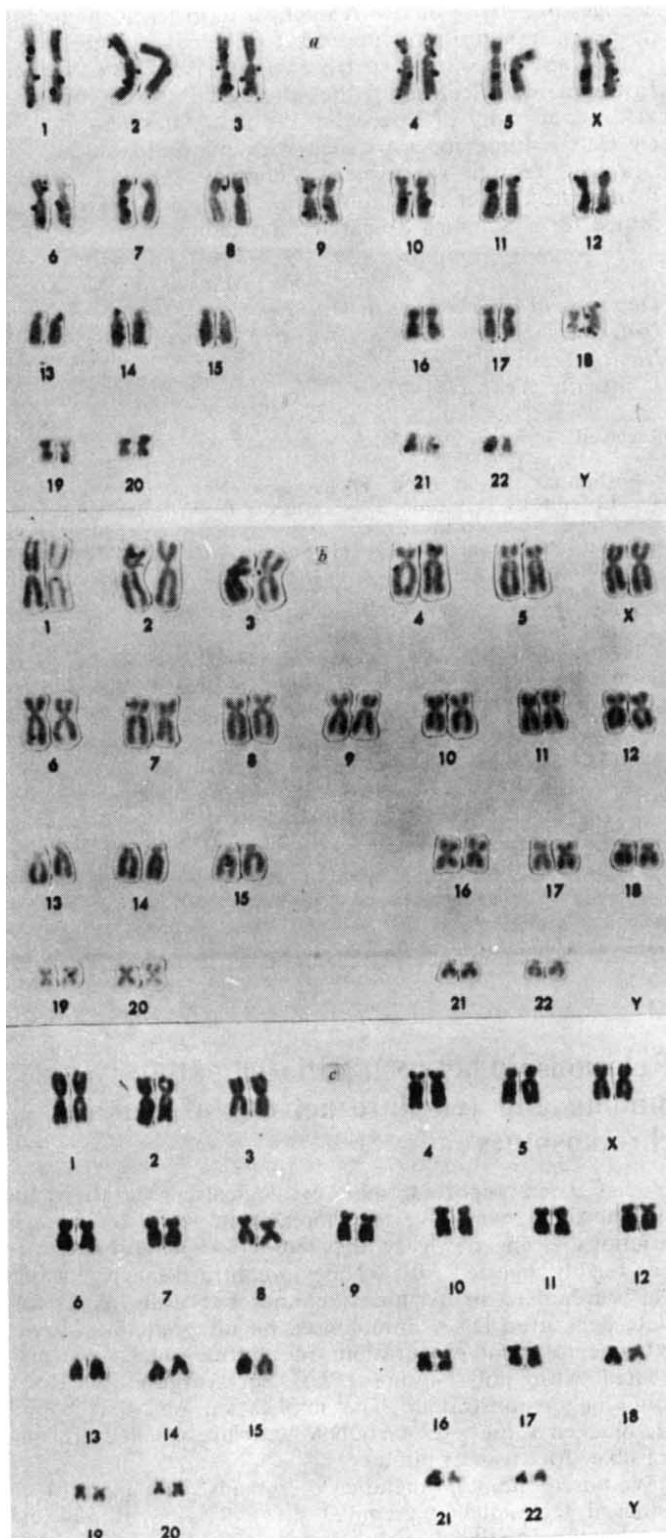


Fig. 1 Typical karyotypes of treated and control human metaphase chromosomes. *a*, Chromosomes treated with tritiated poly-L-lysine ($^3\text{H-PL}$); *b*, chromosomes used as templates in RNA polymerase reaction; *c*, chromosomes subjected to conditions of RNA polymerase reaction without the polymerising enzyme. Tissue culture of human circulating lymphocytes and the preparation of metaphase spreads was done according to a standard procedure³. Slides containing chromosomes were treated with $^3\text{H-PL}$ (molecular weight 50,000–100,000, specific activity 3.32×10^3 c.p.m. mg^{-1}) as follows. 0.1 ml of 0.1% $^3\text{H-PL}$ solution in 0.1 N acetic acid was applied and the slide was covered with a cover slip. After 20 min at room temperature the cover slip was removed, the slide washed with water and dried. After exposure to NTB-2 emulsion for 7 d, the slides were

Table 1 A comparison of $^3\text{H-poly-L-lysine}$ binding, RNA synthesis and quinacrine fluorescence of human A group chromosomes

Chromosome segment	$^3\text{H-PL}$ binding		RNA synthesis		Quinacrine fluorescence
	$\bar{G}$	ΔG	$\bar{G}$	ΔG	
A ₁ e	9.33	-8.87	0.48	1.30	3.20
A ₁ m	4.12	1.40	0.40	0.20	40.00
A ₁ c	13.22	-9.43	—	—	3.20
A ₂ es	15.00	-10.87	—	—	1.20
A ₂ ms	8.34	6.80	1.00	-1.00	16.40
A ₂ c	12.29	14.80	—	—	4.10
A ₂ ml	9.57	-0.50	0.37	—	32.7
A ₂ cl	24.50	-42.87	3.20	-12.00	1.20
A ₃ c	11.38	-8.33	0.25	—	4.4
A ₃ m	4.18	1.93	0.13	0.40	53.9
A ₃ c	9.42	2.87	1.95	—	(7.2)

Average number of grains per unit weight ($\text{g} \times 10^{-15}$) of a given chromosome segment⁶ ($\bar{G}$) was determined separately for karyotypes containing short, medium or long metaphase chromosomes (Fig. 1). For A₁ and A₃ chromosomes in which short and long arms could not be distinguished, the data are given for the combined two end and two middle segments. Forty-six karyotypes (16 short, 16 medium, 14 long) were analysed for $^3\text{H-PL}$ binding and 34 (11 short, 12 medium, 11 long) for RNA synthesis. The data are averages obtained with the three classes of chromosomes. The probable error computed for several groups of karyotypes belonging to the same class ranged from 15–30% of the mean value. The differences in $\bar{G}$ for each chromosomal segment between chromosomes of different length (ΔG) was also computed ($\Delta G = (G_{\text{long}} - G_{\text{medium}}) + (G_{\text{long}} - G_{\text{short}}) + (G_{\text{medium}} - G_{\text{short}})$) to show the effect of chromosomal contraction on the content and distribution of grains. The integrated intensity of quinacrine mustard fluorescence for different chromosomal segments (given in arbitrary units) was determined by measuring the corresponding areas under the fluorescence curves⁷. The value assigned to A₃c segment may vary since this is a polymorphic region⁴ of A₃ chromosome.

nucleic acids are required. On the other hand, the chromosomal segments with high template activity show little fluorescence after treatment with quinacrine mustard⁸. We also observed that both functions depend on the degree of chromosomal contraction. This effect was especially pronounced for the long arm end segment of A₂ chromosome. It did bind more $^3\text{H-PL}$ and functioned better as a template when the chromosome was in a more contracted form.

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examined under a microscope. The grouping of metaphase chromosomes according to the degree of contraction was based on the measurement of A₁ chromosome (short, 8.3 μm ; medium, 10.0 μm ; long, 12.5 μm). Identification of individual chromosomes was aided by the application of a Giemsa banding procedure after treatment of chromosomes with trypsin^{7,8}. RNA polymerase reaction was done on slides containing metaphase spreads (without any pre-treatment) essentially according to the published procedure¹. $^3\text{H-UTP}$ labelled at C5 of the uracil base (specific activity 41.7 Ci mmol^{-1}) was used for RNA labelling. In the control experiments, all the ingredients of the reaction, except the *E. coli* RNA polymerase (specific activity 240 units mg^{-1} assayed in standard conditions), were present. The slides were analysed for grains as described for $^3\text{H-PL}$ binding.

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Lac repressor can be fused to β -galactosidase

GENETIC analysis of *i* gene mutants which had lost the capacity to bind operator but not inducer suggested that *lac* repressor binds with its amino terminus to *lac* operator¹. Here we will show that the carboxy terminus of *lac* repressor is dispensable: We found fusions between the *i* (*lac* repressor) and *z* (β -galactosidase) genes (Fig. 1) which produce fully active *lac* repressor covalently linked to active β -galactosidase.

Such fusions are found among spontaneous *lac*⁺ revertants of an *Escherichia coli* strain carrying the mutations *i*^u and *z*^{u118}. *i*^u stands for a mutation in the *i* promoter which leads to a ten-fold overproduction of *lac* repressor²; *z*^{u118} is a strongly polar nonsense mutation located near the *lac* operator³. The *lac*⁺ revertants of *i*^u*z*^{u118} fall into two classes: inducible revertants (due to direct reversion or unlinked suppression) and mutants that produce β -galactosidase at a low constitutive level. These last mentioned mutants have all the characteristics of *i* *z* fusion: They can be neither induced by inducer nor repressed by a functioning *i* gene in trans. The amount of β -galactosidase made depends on the nature of the *i* gene promoter. Most of these mutants (101 out of 111) no longer produce functioning *lac* repressor. In these mutants part of the structural *i* gene is deleted. In the extracts of ten mutants, however, we found *in vitro* inducer and operator binding. We concentrated our interest on these mutants: Mapping proved that only two of these ten deletions definitely entered the structural part of the *i* gene and that eight others might end in a region of the *i* gene which is still transcribed but not translated (Fig. 1). The β -galactosidase of most of the strains was more heat labile than β -galactosidase from a wild type strain.

We used antibodies against *lac* repressor (anti-repressor) and against β -galactosidase (anti-galactosidase) to decide whether *lac* repressor was fused to β -galactosidase. There is no cross reaction between these antibodies and antigens; however, both anti-repressor and anti-galactosidase precipitate β -galactosidase and *lac* repressor from extracts of fusion 71-56-14 (Table 1). This result strongly suggests, but does not prove, covalent fusion between *lac* repressor and β -galactosidase.

The pure material we needed to settle the question was obtained in the following way: We used extracts of pro-

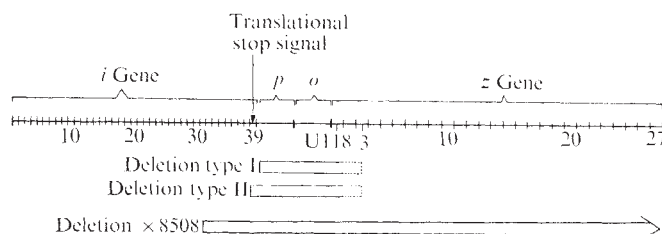


Fig. 1 Genetic map of the *i* gene, the promoter (*p*), operator (*o*), and the *z* gene. The *i* gene is divided in 39 deletion groups⁴, the *z* gene in 27 deletion groups⁵. They are not drawn to scale. Deletion x8630 defines deletion group 39, deletion x8636 deletion group 38 of the *i* gene. Point mutants have been found which recombine with x8630 (Gho, personal communication), indicating that the translational stop signal of the *i* gene falls in deletion group 39. The fusions we found still to have *lac* repressor activity, are either of type I (71-56-9; 71-68-14; 28; 32; 34; 47; 48; 51), or of type II (71-56-14; 71-56-17). Mapping was done as in ref. 4. In some fusions (71-56-14; 17; 71-68-14; 28; 32) *i*^u was crossed out and *i*⁺ back in using deletion x8508 (ref. 6). These *i*⁺ derivatives of the fusion strains were used to see how deep the deletions entered the *z* gene. Those fusions which were tested (71-56-14 and 71-68-14; 28; 32) did recombine with a nonsense mutant mapping in deletion No. 3 but not with another mapping in deletion No. 1.

motor mutant *i*^{a1} (Müller-Hill, unpublished) from one of the strains (71-56-14) as starting material; ammonium sulphate, phosphocellulose and Sepharose 6B were used for purification. We followed both β -galactosidase and repressor activity throughout the purification steps. There is a good correlation, although not a precise one, between the specific activities of *lac* repressor and β -galactosidase throughout the purification (Table 2). The elution profile of the phosphocellulose column shows several fractions which have simultaneously β -galactosidase and repressor activity. The main fraction was further purified on Sepharose 6B. SDS-gels showed that it was at least 90% pure (Fig. 2). The subunit molecular weight is about 155,000 since our protein runs together with the β -subunit of RNA polymerase from *E. coli*. If we assume β -galactosidase to have the most plausible subunit molecular weight of 125,000 (ref. 9) and *lac* repressor a molecular weight of 37,000 (ref. 10), not much has been lost from β -galactosidase and repressor through the fusion. The repressor-galactosidase was derived from a strain that carried a new strong promoter mutation *i*^{a1}. We sequenced the N terminus, using the SDS-dansyl-Edman procedure¹¹ and found it to be identical to the N terminus of *lac* repressor^{1,12}: H₂N-Met-Lys-Pro-Val-Thr-Leu, thereby proving again the covalent fusion and excluding an effect of the promoter mutation *i*^{a1} on the aminoterminal sequence.

Sequence analysis of *lac* repressor¹⁰ had shown that older results¹ with carboxypeptidase B, which seemed to indicate the necessity of an intact C terminus for operator binding activity, were wrong. Our present results show clearly that

Table 1 Results of adding antibodies against *lac* repressor and β -galactosidase in two strains of *E. coli*

strain	anti-repressor added		anti-galactosidase added	
	% <i>lac</i> repressor precipitated	% β -galactosidase precipitated	% <i>lac</i> repressor precipitated	% β -galactosidase precipitated
Φ 71-56-14	35	50	20	55
<i>i</i> ^{a1} <i>z</i> ⁺ (+IPTG)	24	≤ 0.2	≤ 1	50

The fusion 71-56-14 was crossed on the phage λ 80c₁₈₅₇*dlacS*₇ (Φ 71-56-14) to increase the yield of *lac* product after heat induction of a bacterial strain lysogenic for the phage². As comparison, strain *i*^{a1}*z*⁺ grown in the presence of inducer IPTG was used. The heat induction of the fusion strain was carried out as in ref. 2. 0.2 ml of extract were mixed with 0.2 ml of antiserum. The mixture was left overnight at 4° C. The visible precipitates were centrifuged for one minute in an Eppendorf microcentrifuge. The supernatants were discarded. The precipitates were resuspended in 1 ml of TMS buffer⁷ and spun again. The washing procedure was repeated twice. Then repressor and β -galactosidase activity were determined^{7,8}. The values obtained were expressed as percentages of the activities found before precipitation with antiserum.

Table 2 Purification of repressor-galactosidase from strain *i^z* (71-56-14)

Fraction	Total volume (ml)	Total Protein (mg)	Specific activity (% mg ⁻¹ ml)	<i>lac</i> repressor activity		β -galactosidase activity		
				Recovery (%)	Purification	Specific activity (Units mg ⁻¹)	Recovery (%)	Purification
Crude extract	2,200	40,500	4.4	100	1	3,180	100	1
Dialysed ammonium sulphate precipitate (0-35%)	330	5,700	32	100	7	21,600	96	7
Phosphocellulose (main peak)	260	333	146	27	33	29,900	8	9
Sephacrose 6B	84	118	266	17	60	98,000	9	31

400 g of frozen cells were suspended in 600 ml of TMS buffer⁷ and disrupted in a Gaulin homogeniser at 600 kp/cm². The precipitate of a 0-35% ammonium sulphate cut was dissolved in 0.011 M PC buffer (potassium phosphate pH 7.27, 0.5 mM dithioerithrol, 5% glycerol) and dialysed against the same buffer. The dialysed sample was applied to a 500 ml phosphocellulose column (Whatman P 11) equilibrated with 0.011 M PC buffer. The protein was eluted with a linear gradient starting with 1.1 l 0.011 M PC buffer in the mixing chamber and 1.1 l 0.19 M PC buffer in the reservoir. After the gradient the column was first washed with 250 ml of the reservoir buffer, then with 1 l of 0.24 M PC buffer. The main portion of repressor-galactosidase was eluted at a concentration of 0.11 to 0.13 M potassium phosphate. The pooled material from the main peak was concentrated by ammonium sulphate and then passed through Sepharose 6B (column 2.5 $\times$ 100 cm) equilibrated with 0.11 M PC buffer. The protein is eluted in a single peak, containing both *lac* repressor and β -galactosidase activities. The specific *lac* repressor activity of the pure protein was 266: this almost reaches the specific activity of pure *lac* repressor (1,500), if the fourfold increase of subunit molecular weight is taken into consideration.

the C terminus of *lac* repressor is dispensable for activity. Furthermore *lac* repressor functions even with β -galactosidase pinned to its tail.

Both *lac* repressor and β -galactosidase are tetramers. How does the fused protein aggregate? To determine its state of aggregation we analysed the pure material obtained from the sepharose step on a glycerol gradient. Both β -galactosidase and *lac* repressor activity sediment together at the position of normal β -galactosidase (S 16). We also analysed some of the minor fractions from the phosphocellulose column and found them to contain several species of faster sedimenting material. All fractions bind *lac* operator *in vitro*, but only the S 16 fraction binds operator DNA like normal *lac* repressor (Fig. 3). The plot shows that the DNA was 20% active. From the molar ratios of the pro-

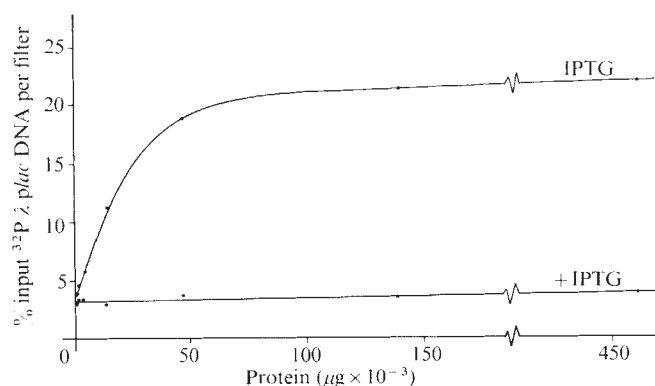


Fig. 3 Bound ³²P- λ plac DNA as a function of the amount of pure repressor-galactosidase in the assay. 3 ml of binding buffer (BB)¹⁴ (without dimethyl sulphoxide), containing the protein, was divided into equal parts and 20 μ l of a 0.1 M IPTG stock was added to one half. Then 0.1 ml of a ³²P- λ plac DNA solution was added and incubated for 8 min at room temperature. The constant amount of DNA in the 1.6 ml assay is 0.43 μ g, with the activity of 23,000 c.p.m. per μ g DNA. Each filter (Sartorius, 0.45 μ m, 25 mm) received 0.5 ml, containing a total input of 3,000 c.p.m. The filters are washed with 1 ml BB each and counted in 10 ml Bray's Solution¹⁵.

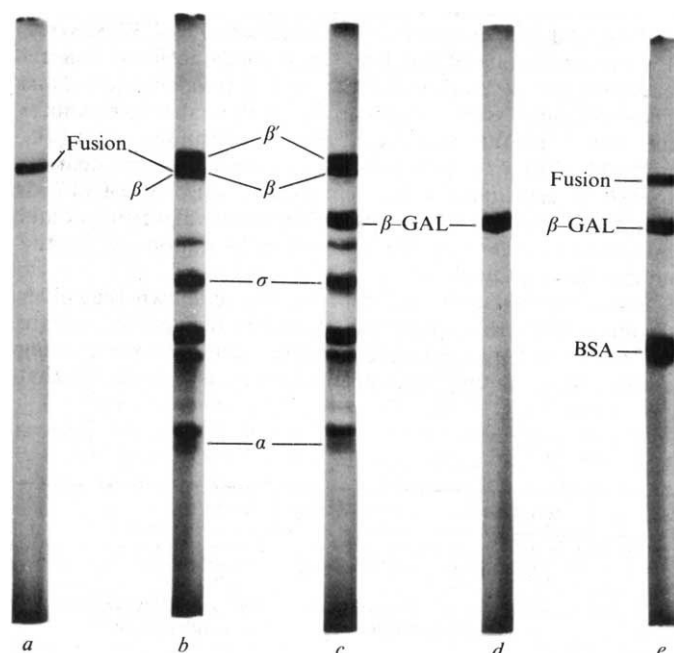


Fig. 2 Determination of the subunit molecular weight of repressor-galactosidase (fusion) by the SDS-polyacrylamide gel electrophoresis. 10 cm 5% acrylamide gels containing 0.1% SDS were prepared according to Weber and Osborn¹³. a, Repressor-galactosidase; b, repressor-galactosidase and *E. coli* RNA-polymerase (Miles-Seravac); c, RNA-polymerase and β -galactosidase; d, β -galactosidase; e, repressor-galactosidase, β -galactosidase, and bovine serum albumin.

tein and DNA we calculate that 20% of the protein is active in DNA binding. The higher aggregates seem also to bind, in a way not understood, to DNA pieces which do not contain *lac* operator.

Finally, we analysed whether homology between *lac* repressor and the N terminus of β -galactosidase is responsible for the relative high frequency of the *i^z* fusions. To test this we measured the frequency of *i^z* fusions in a *Rec A* strain in which recombination is greatly reduced. We found the same number of fusions as in wild type. This excludes sequence homology. Sequence analysis which we have begun should define the unnecessary parts of β -galactosidase and *lac* repressor.

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Mutagenicity of amino acid analogues in eukaryotes

A LOSS of fidelity in DNA replication and repair enzymes in both pro and eukaryotes, is often expressed as a mutation. Examples of this are shown in the mutator gene activity of a DNA polymerase mutant of T4 (ref. 1) and mutants with increased ultraviolet sensitivity or decreased recombination^{2–4}. Springgate and Loeb⁵ have reported the altered fidelity of the DNA polymerase of acute lymphatic leukaemia cells.

Amino acid analogue incorporation into protein has a varying effect on enzyme stability and activity^{6–9} and it seems that the position of the amino acid in the polypeptide is the crucial factor¹⁰.

We have recently reported^{11,12} that the analogues *p*-fluorophenylalanine (PFP) and ethionine (ETH) are strongly mutagenic to reverse mutations in the basidiomycete *Coprinus lagopus*, inducing single base changes with a maximum of a 500-fold increase over the spontaneous mutation frequency shown by 2.5×10^{-4} M DL-PFP which induces 64% growth inhibition. PFP is mutagenic by incorporation into protein, while ETH mutagenesis is a function of transethylation of nucleic acids¹². The mutagenicity of a number of amino acid analogues has been reported in *Chlamydomonas eugametos*¹³ and in *Ustilago maydis*¹⁴.

To gain a better understanding of the mutagenicity of amino acid analogues and because of the availability of defined replication and repair mutants in *Escherichia coli*, we tested the mutagenicity of PFP on reverse mutations in the bacterial system. Two point mutations were studied, the leucine requirement in strain C600 and the tryptophan requirement in strain A58. As with *Coprinus*, PFP was incorporated into the growth medium, in this case liquid Davis minimal medium, at concentrations ranging from 10^{-5} M DL-PFP to 5×10^{-4} M DL-PFP, a concentration which gave linear growth. At time zero, flasks were inoculated with exponentially growing cells and grown to stationary phase or for 5 h in the case of high analogue concentrations. The cells were washed and plated onto minimal medium reversion plates and viability plates supplemented with the auxotrophic requirement. In both cases, however, the analogue PFP had no effect on the reversion frequency of either the *leu* or *trp* loci. The incorporation of ¹⁴C-PFP confirmed that PFP was being incorporated into the protein fraction. Similarly, 10^{-3} M DL-PFP had no mutagenic effect on the reversion frequency of *Pseudomonas aeruginosa* PAO 381 (*leu*⁻).

The mutagenicity of PFP was then tested on bacteriophage T4, chosen for its independent DNA polymerase synthesis. The effect of PFP on the plaque-forming ability of T4rII 271 on *E. coli* K and the plaque-forming ability of the amber mutant T4am122 on the nonsuppressive strain *E. coli* B/r/t, was studied. In both cases phage was inoculated at time zero into a permissive host grown previously for 2 h in the presence of 10^{-4} M DL-PFP. After a further 3 h, growth was stopped and the plaque-forming ability assayed. PFP had no mutagenic effect on either system.

Analogues norleucine and canavanine showed similar negative effects on *E. coli* reversion frequencies. The mutagenicity of ETH was not tested as the strains studied showed resistance to the analogue and ETH is not activated to S-adenosyl-ethionine in *E. coli* (ref. 15).

In *Coprinus*, PFP treatment followed by a 6 min dose from a 6 W Philips TUV mercury vapour lamp (8.54 erg cm^{-2}), showed a maximum mutational synergism of 9.6 times over the expected additive reversion frequencies (P. J. T., and D. L., unpublished). Mutational synergism is indicative of an interaction between mutational pathways; we have interpreted these results as the effect of PFP incorporation into ultraviolet repair enzymes. As PFP showed no mutagenicity in *E. coli* it might be possible to detect its effect by looking for mutational synergism between PFP and ultraviolet treatments; 5×10^{-5} M DL-PFP was chosen as a suitable concentration for pre-ultraviolet treatment, so as not to induce starvation-induced resistance enhancement¹⁶. Log phase cells of *E. coli* C600, grown in the presence of the analogue for 3 h, were irradiated for varying times. Analogue treatment did not affect ultraviolet survival or reversion frequencies of the *leu* locus.

To clarify the difference between pro and eukaryotic responses to analogue mutagenesis, we chose, as a system of comparison, mutations in the mitochondrial and nuclear genes of *Saccharomyces cerevisiae*. The existence of an independent mitochondrial DNA polymerase¹⁷, the selective depression of mitochondrial protein synthesis in the presence of a fermentable substrate, and the differential sensitivity of certain strains to analogues in conditions of growth on nonfermentable or fermentable substrates¹⁸, make the system advantageous for this study.

Two strains of *S. cerevisiae*, D26 (adenine requiring, erythromycin sensitive) and D6 (methionine requiring, erythromycin sensitive) were chosen. D26 showed a three-fold and D6 a two-fold increased sensitivity to PFP on nonfermentable substrate. The effect of PFP on the nuclear markers *ad* and *met* and mutation to erythromycin (1 mg ml^{-1}) resistance—the mitochondrial marker—were studied. Liquid glucose yeast extract and glycerol yeast extract media, supplemented with the analogue at concentrations giving up to 50% growth inhibition were inoculated with 10^6 yeast cells and grown to stationary phase. The cells were washed twice and tested on Wickerams synthetic medium, fully supplemented in the case of viability plates, but without the auxotrophic requirement in the reversion plates. Resistance to erythromycin was tested on glycerol yeast extract with 1 mg ml^{-1} erythromycin added (Table 1).

The positive effect of the analogue on the nuclear genes in yeast, confirms the results in *Coprinus*. The entirely negative effect of the analogue on mitochondrial mutation, if we can

Table 1 The mutagenic effect of PFP on mitochondrial and nuclear markers in *Saccharomyces cerevisiae* strains D26 (*ad*⁻ *eryth*^s) and D6 (*met*⁻ *eryth*^s) when supplemented to both yeast extract glucose (YES) and yeast extract glycerol (YEG) media

		Mutation frequency per 10^6 viable cells			
		Control	1.5×10^{-3} MPFP	3.0×10^{-3} MPFP	4.5×10^{-3} MPFP
		YEG and YES	YEG	YES	YES
D26	<i>ad</i> ⁻	3.5	3.3	9.2	23.2
	<i>eryth</i> ^s	24.6	25.8	13.5	17.6
	<i>met</i> ⁻	3.0	5.9	76	—
D6	<i>eryth</i> ^s	24.3	10.4	13.1	—

compare the mitochondrial chromosome with the chromosome of prokaryotes, confirms the negative results in prokaryotes.

It might be possible to explain the differential response to PFP on the basis of amino acid composition of DNA replication and repair enzymes in pro and eukaryotes, that is, while phenylalanine might be strategically placed in fungal replication and repair enzymes, such as at the active site, phenylalanine differently placed in bacterial and mitochondrial replication and repair enzymes, could account for the difference. From Table 1, the maximum mutagenic effect obtained in yeast is considerably lower than in *Coprinus*, at comparable growth inhibitory concentrations. This can also be explained on the differential importance of phenylalanine in the active centres in the DNA replication and repair enzymes in yeast and *Coprinus*. If this is so, then certain analogues should show mutagenicity in prokaryotes. The few we tested did not. Pardee and Prestidge¹⁹ reported that no mutagenic action of 7-azatryptophan was noted in mutation to streptomycin resistance in *E. coli* or the plaque-forming ability of T2.

The differential response might implicate the presence of histone and nonhistone proteins in eukaryotic chromosomes. PFP induces haploidisation inversions and transversions in certain fungal species by affecting chromosomal division²⁰ and integrity²¹. In *Chlamydomonas* the phenylalanine analogue 2-amino-3-phenylbutanoic acid, as well as being mutagenic, causes chromosomal aberrations, suggested by McBride and Gowan¹³ to be the result of incorporation of the analogue into chromosomal associated proteins. Hare²² reported that canavanine incorporation into histone and nonhistone proteins in mice, mammalian and hamster tumour cells, inhibited DNA synthesis. Chromosomal aberrations and an exaggerated response to mutagens have been found in children suffering from severe protein starvation²³.

Several reports have been made of the differential response of bacteria and higher organisms to various mutagens²⁴⁻²⁷. Most of these effects have been attributed to physiological differences between pro and eukaryotes. The differential response to amino acid analogues indicates a difference on the basis of the replication and repair of DNA.

We thank Dr B. A. Bridges for pointing out to us the work of George and Witkin²⁸ suggesting that some ultraviolet repair enzymes might be inducible by ultraviolet. If this is true then the interpretation of our experiments with PFP and ultraviolet light is more uncertain. This does not, however, alter the validity of the comparison between *C. lagopus* and *E. coli*.

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Naturally occurring cytotoxic anti-tumour antibodies in sera of congenitally athymic (nude) mice

It has been suggested that a natural function of the immune system is the detection and elimination of nascent autochthonous tumours and that clinical tumour development reflects a failure of this immune surveillance function¹. Evidence for the involvement of thymus dependent immune processes in the prevention of spontaneous malignant disease is lacking. Thus, no significant increase in tumour incidence has been observed in either neonatally thymectomised (R. T. Prehn, personal communication) or congenitally athymic (nude) mice^{2,3}. If, as suggested by other studies, immune surveillance does, in fact, operate⁴, it is likely that it will involve a thymus-independent immune process. We have detected in sera of many normal mice IgM antibodies which are cytotoxic for several spontaneous and induced tumour cell lines (unpublished). The possible role of these antibodies in immune surveillance is uncertain, but if they are a manifestation of active immune surveillance, they would be expected also to occur in the sera of nude mice.

Nude mice (from Olac Ltd, Southern Bicester, England) have been studied in this laboratory and shown to have a near complete absence of θ -positive thymus-derived lymphoid cells⁵. Sera were obtained from these mice and were stored in aliquots at -20°C . The sera were tested for complement dependent cytotoxic activity against several tumour lines maintained in this laboratory. Two tumours which recently arose spontaneously in normal C3H mice were included in this study. One is a lymphosarcoma with surface bound IgG₂ immunoglobulin and is referred to as γG2LS . The other is an *in vitro* cell line derived from an ovarian teratoma. The cells are relatively undifferentiated epitheloid cells and are designated Ter-B. The other tumours tested are maintained in ascites form and are used extensively in several laboratories. They are C1300, a neuroblastoma of strain A mice; and EL-4, L1210, and PU-5, leukaemias of C57BL, DBA/2, and BALB/c mice, respectively.

Individual sera obtained from six, ten-week-old nude mice were tested undiluted against various tumour cell lines and were found to possess significant complement dependent lytic activity against all but the PU-5 target cells. A pool of the sera from the individual nude mice was also tested at several dilutions against the different tumour cell lines. The results of these cytotoxic assays are depicted in Fig. 1. The observed levels of cytotoxic activity are within the range of variation obtained when different sera of normal mice are similarly tested (unpublished). Sera from nude mice show no cytotoxic activity when tested on normal allogeneic spleen cells.

The presence of naturally occurring cytotoxic antibody in the sera of nude mice indicates the capacity of the thymus-independent immune system to respond immunologically to

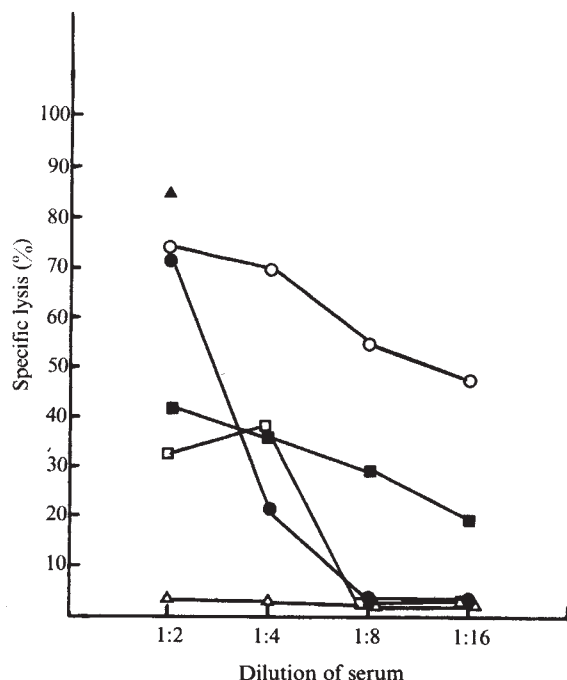


Fig. 1 Complement dependent antitumour cytotoxic activity of serum obtained from nude mice. Target cells were obtained from the following tumours: Ter-B (▲); γG2LS (○); L1210 (●); C1300 (■); E1-4 (□), and PU-5 (△). See text for description of tumours. In each assay, 50 μl of a suspension containing 3×10^6 tumour cells ml⁻¹ in Dulbecco's modified minimal essential medium with 10% foetal calf serum were added to 50 μl of a dilution of pooled serum obtained from nude mice. The mixture was incubated for 45 min in a humidified incubator at 37° C with a 10% CO₂:90% air atmosphere. The cells were washed with 300 μl of medium and resuspended in 50 μl of a 1:6 dilution of rabbit serum as a source of complement. The rabbit serum had been previously absorbed at 2° C with a one-fifth volume of mouse spleen cells to reduce its toxicity. After 45 min incubation in the presence of complement, the tubes were placed in an ice bath. A volume of 50 μl of trypan blue solution was added to the cell suspensions and aliquots removed for microscopic enumeration of % nonviable (stained) cells. Two groups of controls were included in each experiment: first, incubation of tumour cells with serum of nude mice, but followed by the addition of medium rather than complement and second, initial incubation of the cells in medium followed by incubation in complement. Less than 5% nonviable cells were observed with the first group of controls for each tumour cell line tested. Less than 15% nonviable cells were observed with the complement control (C) for each of the tumour cell lines tested. Specific lysis was calculated by subtracting C from the % nonviable cells observed with serum plus complement, dividing by (100-C) and multiplying by 100. Duplicate determinations of cytotoxic activity of nude mouse sera were made for each tumour cell line.

tumour-associated antigens. The nature of the antigens recognised by naturally occurring antibodies in sera of normal mice is currently being investigated. Some of these antigens are expressed in appreciable amounts during foetal development, while others are expressed in certain allogeneic strains^{6,7}. It is possible that cytotoxic anti-tumour antibody production is partly maintained in normal and nude mice by the derepression of these foetal and alloantigenic components on nascent autochthonous tumours.

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Homology between human breast tumour RNA and mouse mammary tumour virus genome

SEVERAL studies have provided indirect evidence linking particles related to mouse mammary tumour virus (MuMTV) with human breast cancer¹⁻³. In addition to morphological and biochemical similarities between human milk particles and known oncornaviruses shown in several laboratories⁴, Axel *et al.*⁵ demonstrated partial homology between MuMTV genome and polysomal RNA isolated from human breast tumour by molecular hybridisation experiments. Using Cs₂SO₄ density gradient analysis they showed that 67% out of 29 tumours tested had detectable amounts of MuMTV-related RNA. But because of intrinsic problems involved in Cs₂SO₄ gradient analysis it is not possible to determine hybridisation quantitatively. In this paper we describe hybridisation experiments using MuMTV-specific DNA probes and human tumour RNA under stringent conditions.

We have used single-strand specific nuclease (S₁) from *Aspergillus oryzae*⁶ for the detection of hybrid formation. This enzyme specifically degrades single-stranded nucleic acids but not double stranded nucleic acids or DNA-RNA hybrids. Highly radioactive, complementary and predominantly single-stranded DNA synthesised by endogenous RNA-directed DNA polymerase of MuMTV in the presence of actinomycin D was used as a probe to detect MuMTV-related sequences in whole cell RNA isolated from human tumours. Most of the human lesions were carefully selected by examining representative frozen sections histologically to give maximum ratio of malignant cells to stromal cells. Detailed data on the histopathology and antigenicity of the tumour tissues in relation to hybridisation will be published elsewhere.

Optimum hybridisation conditions described by Varmus *et al.*⁷ were used (see Table 1 for details). The experiments were performed under conditions of excess RNA and limiting DNA probes. Under these conditions the rate of hybridisation is determined by the concentration of homologous RNA and time of incubation. The product of RNA (mol l⁻¹) and time of incubation (s) is designated *C₀t* which is similar to the *C₀t* value described by Britten and Kohne⁸. The *C₀t* values were computed from the *A₂₆₀* of the RNA samples⁸ and corrected to 0.18 M[Na⁺] according to Britten and Smith⁹.

To provide a rapid determination of hybridisation, tumour RNAs were incubated with MuMTV DNA probe at *C₀t* values greater than 10⁴. Table 1 summarises the results obtained. Any tumour RNA that hybridised more than 10% of the DNA probe was considered significantly positive. It can be seen that out of 17 tumours tested 5 had RNA sequences homologous to MuMTV genome. In one case (HuT 11) the tumour tissue itself revealed some homologous RNA but surrounding 'control' tissue was negative for MuMTV-related RNA sequences. When the DNA probe synthesised by Mason-Pfizer monkey virus

Table 1 Hybridisation of human tumour RNA with MuMTV DNA probes

Tissue	Histopathology	Hybridisation with MuMTV probe	
Human tumour no.		% DNA hybridised	C_{it}
<i>Invasive carcinoma of breast</i>			
HuT-3	Mucinous type	0.89	2.87×10^4
HuT-4	NST*	0.0	2.32×10^4
HuT-5	NST	1.56	3.73×10^4
HuT-6	NST	0.0	2.72×10^4
HuT-8	NST	0.67	3.35×10^4
HuT-9	Medullary type with <i>in situ</i> foci	42.0	2.21×10^4
HuT-10	Medullary type	6.9	1.4×10^4
HuT-12	NST	76.7	6.73×10^4
HuT-13	NST	54.4	3.56×10^4
HuT-16	NST	0.0	3.96×10^4
HuT-17	NST	0.0	2.3×10^4
<i>In situ, carcinoma of breast</i>			
HuT-7	Lobular type	68.0	2.16×10^4
HuT-11	Lobular type	18.0	2.72×10^4
HuT-14	Ductular type	1.66	2.16×10^4
HuT-15	Ductular type	0.0	3.93×10^4
<i>Other human tumours</i>			
HuT-1	Adenocarcinoma of colon	0.89	3.07×10^4
HuT-2	Carcinoma of cecum	4.58	3.34×10^4
<i>Benign breast lesions</i>			
'Control' of HuT-11	Lobular & ductal hyperplasia	0.0	1.19×10^4
'Control' of HuT-14	Lobular & ductal hyperplasia	3.54	1.92×10^4
<i>Controls</i>			
Rat liver	—	0.0	3.0×10^4
MuMTV 70S RNA	—	100	4.12×10^0
RIII lactating mammary gland	—	100	5.00×10^3
C57 transplanted mammary tumour	—	100	2.22×10^4

*NST = No special type

RNA from tissues was isolated by SDS-phenol extractions, DNase treatment and ethanol precipitation as described by Varmus *et al.*⁷ and resuspended in 0.02M Tris HCl pH 7.2 to give a concentration of about 10 mg ml⁻¹. The DNA probes were synthesised from ~1.5 mg purified MuMTV from RIII mouse milk. The following solutions were added to the virus suspended in 200 μ l 0.01 M Tris HCl pH 8.3: 24 μ l of 1 M DTT; 20 μ l of 1% bovine serum albumin; 12 μ l of 5% Nonidet-P40; 16 μ l of 2.5 mM ATP; 50 μ l of 1 mg ml⁻¹ actinomycin D; 100 μ l of a mixture containing 0.25 M Tris-HCl pH 8.3, 0.05 M NaCl, 0.04 M MgCl₂; 60 μ l of a mixture containing 0.01 M each of dCTP, dATP and dGTP; and ³H-TTP (specific activity 50 Ci mmol⁻¹) was added to give a concentration of 10 μ M. After the incubation at 37° C for 18 h the reaction was stopped by adding 1/10 volumes of 10% SDS and 4M NaCl. The reaction mixture was diluted to 2 ml with a buffer containing 0.01 M Tris HCl pH 8.3, 0.01 M EDTA and 0.1 M NaCl and extracted three times with phenol-cresol (7:1 pH 7.5). Following overnight ethanol precipitation the template RNA molecules were digested by treating the probes in 0.003 M EDTA with 100 μ g ml⁻¹ RNase A at 37° C for 1 h. The RNase was destroyed by the treatment with 0.6 M NaOH at 37° C for 1 h (ref. 12). After neutralisation with HCl the probes were precipitated in ethanol and resuspended in 0.02 M Tris HCl pH 7.2. The hybridisation reactions were carried out at high C_{it} values (see text) in duplicate 5 μ l mixture sealed in capillary tubes and incubated at 68° C for 66 h. The mixture contained 30 to 50 μ g RNA and 300–500 c.p.m. of DNA probe in 0.04 M Tris pH 7.2 0.002 M EDTA and 0.6 M NaCl. After the incubation hybridisation mixture was treated with single-strand specific nuclease (S₁) from *Aspergillus oryzae*⁸ according to the method described by Varmus *et al.*⁷. The probes annealed with heterologous RNA (rat liver) were also treated with S₁ to give self-hybridisation values which were used to correct the results.

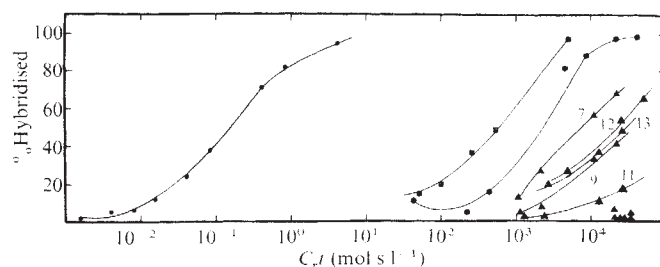


Fig. 1 C_{it} curve analyses of hybridisation between MuMTV DNA probes and RNAs from various sources. The conditions of hybridisation were the same as in Table 1. Different C_{it} values were obtained by diluting the test RNAs with heterologous (rat liver) RNA. ●, MuMTV 70S RNA; ■, RIII lactating mammary glands; ▲, C57 transplanted mammary tumour; ▲, various human tumours including positive tumours (Table 1) HuT 7, 9, 11, 12 and 13.

(MPMV, a virus isolated from the breast tumour of a rhesus monkey) was used for hybridisation under the same conditions, none of the tumours tested showed any RNA sequences homologous to MPMV genome (data not shown).

Hybridisation kinetics for the positive tumour RNAs were observed by C_{it} curve analysis shown in Fig 1. For comparison, C_{it} curves obtained with MuMTV 70S RNA, RNA from RIII lactating mammary glands and from C57 transplanted mammary tumours are also illustrated. All five positive human tumour RNAs showed varied rates of hybridisation. By comparing $C_{it} 1/2$ values of various human tumour RNAs with MuMTV 70S RNA it is possible to arrive at an estimation of virus-related RNA molecules in these tumours. As seen in Table 2, the numbers of MuMTV related RNA molecules per cell in these tumours was low ranging from 1.5 to 8. These estimates are based on the hybridisation of a nonuniformly represented DNA probe and are likely to vary depending upon the representation of viral genome in the synthesised DNA probes.

Thermal stability of hybrids is an important criterion for establishing their true nature. Figure 2 shows a thermal stability experiment performed on the hybrids formed between MuMTV DNA and human tumour RNA. The T_m for the hybrids of MuMTV DNA and RNA from RIII lactating mammary gland (a tissue which produces MuMTV) was 69° C compared with 62° C for the hybrid of MuMTV DNA and human tumour RNA. This difference in thermal stability would indicate about 5% mismatching between the MuMTV DNA and the tumour RNA (ref. 10). Considering the species differences between the sources of two nucleic acids, the hybrids were remarkably well matched.

Extensive electron microscopic searches have failed to reveal unequivocally the presence of complete MuMTV-like

Table 2 Estimations of MuMTV 70S RNA equivalents per cell

Sample	$C_{it} 1/2^*$	% Viral RNA	MuMTV 70S RNA equivalents per cell
MuMTV 70S RNA	1.5×10^{-1}	100	—
HuT 7	7.5×10^3	0.002	8
HuT 9	3.3×10^4	0.00045	1.5
HuT 12	2.1×10^4	0.00072	2.2
HuT 13	2.75×10^4	0.00054	1.68
RIII lactating mammary glands	5.2×10^2	0.029	90
C57 transplanted mammary tumour	1.9×10^3	0.0078	25

* C_{it} at which 50% of the input DNA probe was hybridised. By comparing $C_{it} 1/2$ of MuMTV 70S RNA and of the test RNAs, percentage of MuMTV-related RNA in a given sample was determined. Based on assumptions that each MuMTV 70S RNA weighs 1.6×10^{-11} μ g and total cellular RNA weighs 5×10^{-6} μ g, the number of MuMTV 70S-related RNA molecules per cell was estimated.

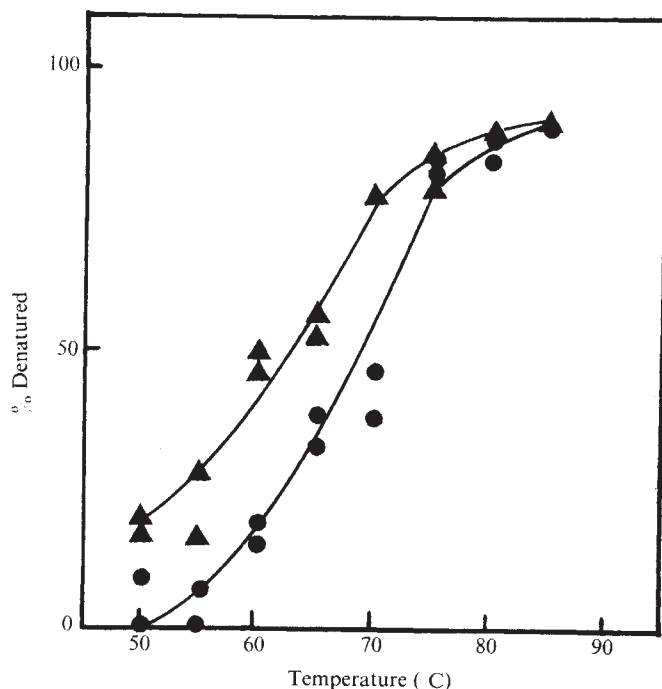


Fig. 2 Thermal stability experiment. Approximately 7,000 c.p.m. of MuMTV probe DNA were annealed with RNAs from human breast tumour (▲) and RIII lactating mammary gland (●). Following ethanol precipitation the hybrids were resuspended in 0.01 M Tris HCl pH 8.3 and aliquots were heated at various temperatures for 15 min, chilled quickly and treated with S_1 nuclease as described in Table 1.

particles in human breast tumours. (A. B. V., unpublished work, and N. H. Sarkar, personal communication.) But the results shown in this communication are very similar to those reported⁷ for murine tissues apparently negative for MuMTV production. Particularly, in spontaneous mammary tumours of BALB/c mice, B-particles of MuMTV are not seen (N. H. Sarkar, personal communication), but a small amount of MuMTV-related RNA is present^{7,11}.

According to current theories of viral oncogenesis, the genome of the virus should be integrated with the host genome. If a MuMTV-related virus is involved in the aetiology of human breast cancer, one should expect MuMTV-related genomic information integrated into the DNA of the tumour cells. We plan to investigate this possibility using molecular hybridisation techniques.

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Levamisole inactive in treatment of four animal tumours

LEVAMISOLE (2,3,5,6-tetrahydro-6-phenylimidazo (2, 1-b) thiazole) a compound known to be active against a wide range of nematodes^{1,2}, has been reported to enhance the humoral and cellular immune responses of animals and to reduce the incidence of both primary tumours and lung metastases in the Lewis lung (3a) tumour system in C57BL mice³. We report here studies on the effect of levamisole on the growth of four transplantable tumours, all known to be immunogenic. They were: a transplantable adenovirus 12-induced tumour of CBA mice⁴; a chick embryo lethal orphan (CELO) virus-induced tumour of hamsters⁵; a Moloney leukaemia virus-induced lymphoma of BALB/c mice⁶; and a chemically-induced metastasising anaplastic neoplasm of inbred rats⁷. We also studied the effect of levamisole on the humoral antibody response to influenza virus vaccine in hamsters.

The tumours were maintained by serial, subcutaneous inoculation of tumour fragments at 2–3 week intervals. For inoculation of experimental animals, small tumours of 10–15 mm diameter were removed from the animals, freed from normal and necrotic tissue and washed with Hank's saline. They were then minced to small fragments and treated with 0.25% trypsin; the resulting single cell suspension was washed three times in Hank's saline, and suspended to the required concentration in Eagle's minimal essential medium containing 2% inactivated calf serum. The cells were used immediately to inoculate animals. A 10 mg ml⁻¹ solution of levamisole was made in pyrogen-free saline, and stored at –20°C. Immediately before use, aliquots of the stock solution were thawed, and diluted to the required concentration in pyrogen-free saline.

Hamsters were inoculated subcutaneously with 5×10^5 viable adenovirus 12-induced tumour cells in an 0.2 ml volume. One group of these animals was inoculated subcutaneously with 0.5 mg kg⁻¹ of levamisole from 4 d before inoculation with tumour cells, a second group was inoculated with levamisole from the day of tumour cell inoculation, and a third group was untreated. Levamisole was given every 48 h from the start of treatment to the end of the study. The hamsters were examined regularly for tumours, and the diameter of tumours were measured with calipers. The results are shown in Table 1. Of the 10 hamsters which were not treated with levamisole, a single tumour was observed 17 d after inoculation, and three animals had large tumours (>2.5 cm in diameter) 35 d after inoculation. In contrast, the incidence of tumours for hamsters which had been treated with levamisole from 4 d before tumour

Table 1 Effect of levamisole on transplanted adenovirus 12-induced tumours of CBA mice

Levamisole from day (0.5mg kg ⁻¹)	10	No. of tumours (day after inoculation)				
		14	17	24	31	35
-4*	0/10	1/10†	4/10 (0.25)	5/10 (1.1)	7/10 (1.8)	7/10 (> 2.5)
0	0/10	3/10 (0.1)	5/10 (0.5)	6/10 (1.3)	9/10 (1.9)	9/10 (> 2.5)
Nil	0/10	0/10	1/10 (0.5)	3/10 (1.0)	3/10 (2.1)	3/10 (> 2.5)

* Treatment (0.5 mg kg⁻¹) subcutaneously on alternate days from 4 d before tumour cell inoculation to end of study.

† No. animals with tumours / No. animals inoculated (mean tumour diameter in cm)

Table 2 Effect of levamisole on transplanted Rd/3 tumour cells of rats

Experiment no.	Levamisole from day (0.5mg kg ⁻¹)	Incidence of tumours at 35 d after inoculation	popliteal lymph node (grade)	para-aortic lymph node (grade)
		foot pad (grade)*†		
1	-5*	3/3 (3, 3, 3)†	3/3 (2, 2, 1)	1/3 (1)
	Nil	3/3 (3, 3, 3)	3/3 (3, 2, 2)	2/3 (1, 1)
2	0	3/3 (3, 3, 3)	1/3 (3)	1/3 (2)
	Nil	2/3 (3, 3)	2/3 (2, 1)	0/3
3	14	3/3 (3, 3, 3)	2/3 (2, 1)	1/3 (1)
	Nil	3/3 (3, 3, 3)	3/3 (3, 3, 2)	3/3 (3, 3, 2)

* Treatment (0.5 mg kg⁻¹) subcutaneously daily from day 5 before tumour cell inoculation to the end of the study.

† No. animals with tumours / No. animals inoculated (grade of tumour growth).

‡ Grade 1, tumour cells present only in subcapsular sinus; grade 2, tumour cells present in subcapsular and radial sinuses, but without destruction of the lymph node; grade 3, massive replacement of the lymph node by neoplasm.

Table 3 Effect of levamisole on HI antibody response to influenza A2/Aichi/68 vaccine in hamsters

Previously infected with A1/FM/1/47	A2/Aichi/68 vaccine given (150 CCA)	Levamisole* treatment (0.1 mg kg ⁻¹)	Serum HI antibody to (following immunisation with 150 CCA of A2/Aichi/68 vaccine)		
			A1/FM/1/47 21 d post infection	14 d post immunisation	A2/Aichi/68 21 d post immunisation
+	+	-	6/6 (240-640)†	1/6 (15)	5/6 (15)
+	+	+	6/6 (160-640)	4/6 (15-60)	5/6 (10-120)
-	+	-	0/6	0/6	0/6
-	+	+	0/6	0/6	1/6 (15)

* 0.1 mg kg⁻¹ inoculated subcutaneously on day of immunisation, and subsequently every 48 h for 14 d.

† No. hamsters with HI antibody / No. hamsters tested (range of HI antibody titres).

cell inoculation was 7/10, and for animals inoculated with the drug from the day of tumour cell inoculation the incidence was 9/10, 35 d after inoculation. The incidence of tumours in the two groups of hamsters treated with levamisole, when taken together, was significantly greater than that for the untreated animals ($\chi^2 = 4.965$; $N = 1$; $P = < 0.02$). Levamisole treatment therefore increased the incidence of transplanted adenovirus 12-induced tumours in hamsters.

In a similar way, the transplantability of a CELO virus-induced tumour of hamsters and a Moloney virus-induced lymphoma of BALB/c mice was investigated in animals treated with levamisole. The incidence of both these tumours was similar in treated and untreated animals. Thus, levamisole treatment did not decrease either tumour incidence or the size of tumours produced by transplanted CELO virus-induced tumour cells of hamsters, or Moloney virus-induced tumour cells of mice.

Groups of rats were inoculated into the foot pad with 5×10^6 RD3 cells; some of these animals were given levamisole (0.5 mg kg⁻¹) daily for 5 d before tumour cell inoculation, others from the day of tumour cell inoculation, and a further group were given levamisole from 14 d after tumour cell inoculation when the tumours were well established. The incidence of tumours at the site of inoculation, and the grade of metastatic tumour growth, is shown in Table 2. For animals treated with levamisole before or from the day of, tumour inoculation, the incidence of tumours in the foot pad and the extent of metastatic tumour growth in the popliteal or para-aortic lymph

glands was similar for treated and untreated control rats (Table 2, Experiments 1 and 2). In the remaining test, where levamisole treatment was started 14 d after tumour cell inoculation, the degree of metastatic spread was less than that of untreated rats (Table 2; Experiment 3). This result, however, could not be repeated in further studies. The results indicate that levamisole did not significantly affect the incidence of primary or metastatic growth of RD3 tumour cells.

Two groups of hamsters were infected intranasally with influenza virus A1/FM/1/47 (H1N1). It has been shown in previous studies that hamsters do not produce haemagglutination inhibiting (HI) antibody in response to conventional doses of influenza virus A2/Aichi/68 vaccine unless previously infected with live influenza A virus^{8,9}. Three weeks after virus infection, these hamsters, which all developed HI antibody to the infecting virus, together with two groups of normal hamsters, were inoculated intramuscularly with 150 CCA of inactivated influenza virus A2/Aichi/2/68 vaccine. One group of infected and one group of uninfected animals were also given 0.1 mg kg⁻¹ levamisole subcutaneously on the day of immunisation, and subsequently every 48 h for 14 d. Serum specimens were collected from all hamsters at each stage of the experiment from the retro-orbital sinus, and tested for HI antibody. The results are shown in Table 3. None of the hamsters given vaccine only produced demonstrable serum HI antibody and one of the immunised animals given levamisole produced antibody (Table 3). For hamsters infected with influenza virus A1/FM/1/47 and later immunised, one animal

developed serum HI antibody detectable at 14 d after inoculation, and four developed antibody after 21 d. In contrast, for the six virus infected and immunised hamsters which were given levamisole, four developed serum HI antibody to the vaccine virus at 14 d after immunisation (geometric mean titre (GMT) 1:22.8) and one developed HI antibody at 21 d after immunisation (GMT = 1:34.6). Thus, in hamsters primed by previous infection with influenza virus A1/FM/1/47, levamisole promoted an earlier serum HI antibody response, and higher titres of antibody were detected.

The reaction of the host to experimental chemical or virus-induced tumours has been shown to be a cell-mediated immune reaction^{10,11} and this cellular immune reaction has been specifically shown for the adenovirus 12-induced tumour of CBA mice and CELO virus-induced tumours of hamsters used in the present study (ref. 12 and R.C.R., and C.W.P., unpublished). In addition, factors which promote the cellular immune reaction of the host may result in an increased immunity to transplanted tumour cells¹³⁻¹⁵. Levamisole has been reported to increase the immune response of mice; the drug re-established the immune reactions of aged mice, and enhanced cell-mediated immune reaction. In addition, levamisole was reported to cause a significant reduction in primary tumour formation and metastases in mice inoculated with a transplantable lung tumour³. In our study, however, the effect of the drug on tumour development could not be extended to four further transplanted animal tumours. Levamisole, at the same concentration as used in the earlier study, had no effect on three transplantable tumours; and the effect on an adenovirus 12-induced tumour of CBA mice was to enhance tumour development.

Sera from CBA mice bearing transplanted adenovirus 12-induced tumours has been shown to contain a serum factor which promoted the development of transplanted tumours by interfering with cell-mediated immunity¹². Blocking factor has been described for other tumour-bearing animals^{16,17} and in serum from patients with cancer. It has been suggested that the blocking factor is a serum antibody¹⁸ or antigen-antibody complex¹⁹. Thus, the promotion of humoral antibody may lead to the formation of blocking factors which would enhance tumour growth.

Our study shows that levamisole enhanced the serum HI antibody response of hamsters to A2/Aichi/68 vaccine, and lack of activity to this drug may be due to the stimulation of humoral antibody and the blocking of cell-mediated immunity. In contrast, levamisole was shown to reduce the incidence of Lewis 3a tumours of mice³. The effect of levamisole may vary for different tumour systems, therefore, as the antigenicity of tumours and the immune response to different tumours is not identical.

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Embryo-derived teratocarcinomas elicit splenomegaly in syngeneic host

MOUSE egg-cylinders transplanted to extrauterine sites develop either as teratomas (benign tumours composed of adult tissues) or as teratocarcinomas (retransplantable malignant tumours containing, in addition, undifferentiated embryonic carcinoma cells)¹⁻⁴. Rat egg-cylinders similarly transplanted, however, always develop as teratoma⁵. When transplanted to a syngeneic host, C57BL mouse egg-cylinders develop as teratomas but when transplanted to (C3H × C57BL)F₁ hybrids they develop into teratocarcinoma^{3,4}. This suggests that the host reaction is important in determining the fate of a transplanted embryo. Specific embryonic and foetal antigens which are re-expressed during malignant transformation are now well established⁶⁻⁸, and we thought that an immunological reaction of the host against embryonic antigens in the egg-cylinder might determine whether the transplant develops as teratoma or teratocarcinoma. Splenomegaly is considered to be a sign that a host has reacted to the antigenic stimulation of a graft or tumour^{9,10}, and we have found that although it is always present in mice with transplanted egg-cylinders, it is much more pronounced in those with teratocarcinoma. Splenectomy, however, reduced the growth of the transplanted embryos, suggesting that the immunological reaction of the host stimulates the growth of the graft.

Seven-day-old mouse egg-cylinders (the day when the vaginal plug was found was considered day 0) were isolated from uterine swellings of pregnant C3H/H mice and transplanted under the kidney capsules or into the testes of 6-8-week-old syngeneic or (C57BL × C3H/H)F₁ hybrid recipients as before^{1,3}. To exclude reaction to antigens linked to the Y chromosome¹¹, and presumably present in half of the transplanted egg-cylinders, all recipients were males. Animals were killed two months later. Tumours grown from the embryos and the spleen of each tumour-bearing animal were weighed and examined histologically. Male animals of the same age kept under identical conditions served as controls for the weight of the spleen. Some of the C3H/H animals were splenectomised at the time of grafting.

As Table 1 shows, the weight of the tumours obtained from under the kidney capsule differed significantly from those obtained by grafting the embryo into the testis. Tumours from embryos grafted to hybrid recipients weighed significantly more than those in syngeneic hosts. Tumours from embryos grafted into syngeneic hosts which were splenectomised at the time of transplantation were much smaller and weighed significantly less than tumours

Table 1 Weight of tumours grafted to the testis and under the kidney capsule

Location	Strain of recipient		
	C3H/H	(C3H/H × C57BL) F ₁ hybrid	C3H/H splenecto- mised
Kidney	3,212 ± 689 (31)	4,130 ± 805 (29)	1,139 ± 449 (21)
Testis	2,149 ± 529 (39)	—	—

All tumours were obtained from grafts of 7-d-old C3H/H mouse egg-cylinders. Weight is given in mg ± s.e. When the *t*-test was applied, values for each group differed significantly from all others ($P < 0.01$). The number of animals in each group is given in parentheses.

in untreated syngeneic hosts, irrespective of the site of transplantation.

All grafts induced some increase in the weight of the host's spleen (Table 2). Embryo-derived teratoid tumours were classified on a histological basis into benign teratomas and teratocarcinomas. Spleens were significantly heavier in animals bearing teratocarcinomas than those bearing teratomas. Tumours obtained from under the kidney capsule induced significantly more splenomegaly than those in the testis. The difference in the weight of spleens is not only statistically significant when all tumours in the kidney are compared with tumours in the testes, but also when teratomas are compared with teratomas and teratocarcinomas are compared with teratocarcinomas in the two locations. In spite of the larger size of tumours obtained under the kidney capsule in hybrid hosts compared with those in syngeneic hosts (Table 1), there was no difference in the weight of spleens in the two groups (Table 2).

Table 2 Weight of spleens in mice bearing embryo-derived teratomas and teratocarcinomas

Location	Strain of recipient			
	C3H/H		(C3H/H × C57BL) F ₁ hybrid	
	Teratoma	Terato- carcinoma	Teratoma	Terato- carcinoma
Kidney	194 ± 30 (16)	374 ± 37 (15)	162 ± 37 (13)	353 ± 37 (16)
Testis	132 ± 3 (23)	276 ± 23 (16)	—	—
Control	117 ± 2.45 (18)		122 ± 3.22 (15)	

Values are given in mg ± s.e. The *t*-test was used to compare means. All values differed significantly from the controls ($P < 0.01$) as well as among themselves ($P < 0.01$) except for the weight of spleens in C3H/H and F₁ hybrid mice grafted with embryos under the kidney capsule. All tumours were derived from C3H/H egg-cylinders. The number of animals in each group is given in parentheses. Tumours containing undifferentiated embryonic cells (embryonic carcinoma cells) were classified as teratocarcinomas. Spleens from normal untreated males served as controls.

Our data indicate that the weight of the spleen increases in all animals bearing grafted embryos. This splenomegaly suggests an immune type of reaction. Recent results have shown a significant increase in the total number of spleen cells, the number of lymphoid subpopulations and in T and B cell functions in tumour-bearing animals¹². Since only the largest grafts contained necrotic tissue, splenomegaly resulting from reaction to tissue destruction could be excluded with a high degree of certainty. Histologically, the appearance of these spleens corresponds to the descriptions of graft-induced splenomegaly^{9,10}.

We found heavier spleens in animals with teratocarcinomas than in those with small, nonproliferating tera-

tomas (Table 2). There are at least two possible explanations for this: one is that there is more tissue for antigenic stimulation available in the large teratocarcinomas than in the usually much smaller teratomas; second, since teratocarcinomas contain foci of undifferentiated embryonic cells, these cells are developmentally and antigenically different from the host, and therefore may exert a stronger antigenic stimulation than the cells in the teratomas.

When transplanted, all grafts contain undifferentiated embryonic cells¹³, but these disappear from teratomas and persist only in teratocarcinomas. We suggest that the slight increase in the weight of the spleen of the teratoma-bearing animal represents a reaction to the presence of undifferentiated embryonic cells during the first 15 d after grafting of the egg-cylinder. Only the persistence of embryonic undifferentiated cells in the teratocarcinomas, however, could induce a three-fold or even more pronounced increase in the weight of the host's spleen.

Whether the size of testicular tumours, which differs as Table 1 shows, is determined by some anatomical factor (blood supply, lower temperatures due to the scrotal location, or tenseness of the tunica albuginea) or is related to a possibly more or less immunologically privileged site for the growth of embryonic tissues remains to be determined.

The greater weight of tumours obtained in hybrids at the same site in syngeneic hosts may be explained by minor antigenic differences between host and graft which provoked a stimulatory immune reaction¹⁴. The reduction in weight of embryo-derived tumours caused by splenectomy at the time of grafting might be explained if blocking antibodies prevent tumour-invoked cell-mediated immunity in intact mice. In splenectomised animals such antibodies could be synthesised in much smaller quantities, leaving embryonic carcinoma cells exposed to the cellular immune response. Observations⁸ that stimulation with foetal cells protects splenectomised hamsters much better than intact hamsters against SV40-induced tumours could be explained by similar mechanisms. Enhancing antibodies¹⁵, secreted by the spleen, and thus not present in splenectomised animals, could also explain the smaller tumour growth. Our finding^{3,4} that about half of the transplanted embryos develop as teratocarcinomas and half as teratomas might mean that small changes in the host's immune response could swing development either way. Tumour cells probably produce many different classes of antibody, so that the outcome of tumour-immune system interaction would depend on many functions not easily controlled during an experiment. We are now trying to characterise the humoral and cellular response elicited by embryo-derived tumour, and to discover how different components of immune response might regulate the development of transplanted embryo.

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Immunisation of marmoset monkeys with a killed oncogenic herpesvirus

HERPESVIRUSES are suspected to be causal agents in human malignant neoplasia. At the present level of knowledge, clinical trials in man with viral vaccines against oncogenic herpesviruses cannot be initiated, but the oncogenic herpesviruses derived from monkeys^{1,2} can be used *in vivo* to study the problems of herpesvirus cancer vaccines. The oncogenic herpesvirus saimiri (HVS) regularly induces malignant lymphoma in cotton topped (CT) marmoset monkeys (*S. oedipus*). A killed vaccine from HVS was developed in our laboratory and tested for safety and efficacy in this primate model system.

For the production of the killed vaccine HVS isolate No. 810 (ref. 1) was cultivated on owl monkey kidney (OMK) cells³.

Table 1 Immunisation of six CT marmoset monkeys with killed HVS

Animal	Time post vaccination (d)	Infectivity titre of whole blood (TCID ₅₀ ml ⁻¹)*	Serum antibodies against HVS	
			NI†	CF‡
174 A	267§	< 10 ^{0.0}	> 3.3	1: 8
175 A	253§	< 10 ^{0.0}	2.5	¶
191 A	211§	< 10 ^{0.0}	3.3	¶
214	122§	< 10 ^{0.0}	> 3.0	1:64
215	122§	< 10 ^{0.0}	3.0	1:64
216	122§	< 10 ^{0.0}	> 3.0	¶

* Determined in OMK cells.

† Neutralisation indices.

‡ Titres of HVS specific complement fixing antibodies.

§ Monkey still alive.

¶ Sera had anticomplementary activity.

Three days before collection, when the cytopathic effect of HVS appeared, the OMK cell cultures were washed and fed with fresh medium, free of serum. The stock virus, stored at 4° C, was centrifuged twice at 2,000g for 10 min and the clarified fluid containing virus was passed through Millipore membrane filters (450 µm). The filtrate was sonicated for 10 s (Branson Sonifier B-12, micro tip, maximal output). The infectivity titre of this preparation, measured in OMK cells, was 10^{5.0}–10^{5.5} TCID₅₀ ml⁻¹. The virus was distributed into 100 ml glass vials and heated at 56° C for 4 h. This heat-treatment completely destroyed the infectivity which was tested in OMK cell cultures. The heat inactivation did not significantly reduce the titre of the HVS specific complement fixing (CF) antigens. CF titres were determined according to standard techniques⁴ using an HVS antiserum from an owl monkey⁵. In addition to the heat treatment the virus preparation was incubated with formaldehyde (100 µg HCHO ml⁻¹, pH adjusted to 6.5 at 37° C). The formaldehyde treatment was stopped after 6 d by neutralisation with equimolar sodium-disulphite (Na₂S₂O₅). The formaldehyde had inactivated 50–75% of the HVS specific CF antigens. The resulting suspension was concentrated 100-fold by dialysis against Aquacide (Calbiochem, Los Angeles). The concentrate was centrifuged at 12,000g for 10 min at 4° C (B 20 centrifuge, International Equipment, Boston) and the supernatant was stored at –60° C and used for vaccination. From each vaccine preparation (10 ml), 30% were tested for cytopathogenicity in OMK cells. No CPE appeared in these cultures during an observation period of 36 d.

In contrast to previously reported findings⁵ small amounts of infectious HVS particles were found in our laboratory when the heat treatment of HVS at 56° C was stopped after 30 min. The survival curve of HVS is multicomponential after treatment with heat as well as after treatment with formaldehyde. For vaccine production this problem could be solved by prolonged incubation periods.

The killed vaccines were tested for infectivity, oncogenicity and antigenicity in CT marmoset monkeys. A distinction has to be drawn between infectivity and oncogenicity of herpesviruses since killed herpesvirus hominis induced neoplastic transformation *in vitro* without productive infection^{6,7}. After being absorbed with Aluminiumhydroxygel (10% v/v of a 3% solution, Behringwerke, Marburg, West Germany) as adjuvant, nine CT marmosets were immunised. Four or five intramuscular inoculations of 1.0 ml each of the killed HVS concentrate were given to each animal within 4–6 weeks. The killed HVS vaccine in all these monkeys induced high titres of neutralising and CF antibodies against HVS (Table 1). The neutralisation tests were performed in OMK cell cultures with a constant serum dilution (1:5) and varying ten-fold virus dilution according to standard techniques⁴. The serum antibodies appeared about 3 weeks after the first inoculation and reached titres as high as those found in the sera of the three non-immunised control monkeys, who developed malignant lymphoma after inoculation of infectious HVS (Table 2). In contrast to the tumour-bearing monkeys (Table 2), HVS could

Table 2 Challenge of three immunised CT marmoset monkeys and infection of three control animals with 10^{3.8} TCID₅₀ of live HVS

Animal	Time interval between immunisation and challenge (d)	Survival time after inoculation of HVS (d)	Infectivity titre of whole blood (TCID ₅₀ ml ⁻¹)*	Serum antibodies against HVS	
				NI†	CF‡
174 B§	53	61¶	< 10 ^{0.0}	> 3.5	1: 8
175 B§	60	64**	10 ^{5.5}	> 3.5	1: 16
191 B§	56	94**	10 ^{6.5}	3.5	1:128
184	Not immunised	33**	> 10 ^{5.5}	3.0	
185	Not immunised	30**	> 10 ^{5.5}	> 3.3	1: 64
186	Not immunised	28**	10 ^{5.0}	3.0	

* Determined in OMK cells at the time of death.

† Neutralisation indices at the time of death.

‡ Titres of HVS specific complement fixing antibodies at the time of death.

§ Animals immunised in the same way as animals 174 A, 175 A and 191 A (Table 1).

¶ Died without signs of a tumour.

** Died of malignant lymphoma.

not be isolated from the immunised animals by cocultivation of fresh peripheral blood with OMK cells (Table 1). The vaccines proved not only to be free of infectious HVS, but also to be non-oncogenic. All of the immunised monkeys remained clinically well; two of them (174 A and 175 A) have been now under observation for 9 months (Table 1).

The immunised monkeys 174 B, 175 B and 191 B were challenged with $10^{3.8}$ TCID₅₀ of cell-free HVS (passed through a 450 μ m Millipore filter) by intramuscular inoculation. Monkey 174 B died 61 d after infection, of an accident, without signs of malignant lymphoma. Animals 175 B and 191 B died 64 and 94 d post infection of a tumour which was characteristic for HVS in gross pathology and histopathology (Table 2). Three control monkeys who received the same HVS inoculation had already died 28, 30 and 33 d post infection because of malignant lymphoma (Table 2). This result indicates that the immunisation delayed the development of malignancy. Experiments are in progress to examine whether CT marmoset monkeys, actively immunised against HVS, are resistant to challenge with smaller doses of HVS than the very high challenge dosage already examined here.

These findings clearly demonstrate that a killed vaccine from oncogenic HVS can be prepared which is safe in respect to infectivity and oncogenicity. The immunisation of CT marmoset monkeys with the killed vaccine induced high titres of HVS specific serum antibodies and delayed tumour development after challenge with a very large dose of infectious HVS. The recent demonstration of induction of lymphomas in CT marmoset monkeys⁸ and an owl monkey⁹ by EB virus may aid in the extrapolation of our data in regard to the wanted vaccine against Burkitt's lymphoma in man.

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Involvement of IgE in con A-induced histamine release from human basophils *in vitro*

CONCAVALIN A (con A) induces the release of histamine from rat¹ and hamster mast cells² and human basophils. Time course, cationic requirements, enzymatic sequences and dose-response seem to be the same for con A-induced release as for antigen-induced release, which is mediated by reaginic antibody (reagin), believed to be bound by its Fc portion to a receptor in the membrane of the histamine-releasing cell. Human reagin is an antibody of the IgE class³. Antibodies⁴ specific for IgE can induce histamine release whereas the papain digest⁵ of those antibodies (which produces monomer anti-IgE) fails to elicit a response and can inhibit the intact anti-IgE-induced release⁶. The requirement for bridging to

initiate histamine release has been established⁷⁻¹³. The types of sugar moieties to which con A binds have been studied¹⁴⁻¹⁷, and the physicochemical properties of con A^{15,18-22} indicate that at physiological pH it can bridge two or more of those sugar moieties. The question is raised as to whether con A is bridging sugar moieties at the receptor for reagin, or whether it is bridging sugar moieties which are directly attached to the reagin. The nature of the receptor remains to be established and the observation that con A can induce histamine release raised expectations that this interaction could elucidate some of the properties of this receptor.

The premise that con A is interacting with a receptor has been weakened by the report¹ that mast cells from rats immunised to produce high reaginic antibody released to con A whereas mast cells from nonimmunised rats did not. I report here that con A release from human basophils involves IgE. Antibodies specific for the Fc portion of IgE (Pharmacia) and antibodies specific for the Fab portion of human immunoglobulins (Behring) were used. They were papain-digested to a monomer form, and lost the ability to induce histamine release but not the ability to bind the IgE. The data show that release induced by con A (Pharmacia) can be inhibited by Fc-specific monomer anti-IgE but not by monomer anti-Fab (Fig. 1). This indicates that con A-induced release in human basophils is mediated by the Fc, but not the Fab, portion of the membrane-bound IgE. The fact that Fc-specific monomer anti-IgE-inhibited release is not conclusive evidence that con A is not bridging sugar moieties at the receptor for IgE. If the IgE receptor has sugar groups close to the Fc portion of IgE, then a steric hindrance of the con A by the monomer is a possibility. However, human basophils can be passively sensitised²³, which implies that not all the receptors for IgE are occupied. It is not likely that the monomer would sterically hinder the binding of con A to an unoccupied receptor. Since inhibition by the monomer is nearly complete, it indicates that either the receptor has no sugar moieties to which con A binds, or that the receptor must have an IgE molecule in place before an activation of the basophil can be mediated by it.

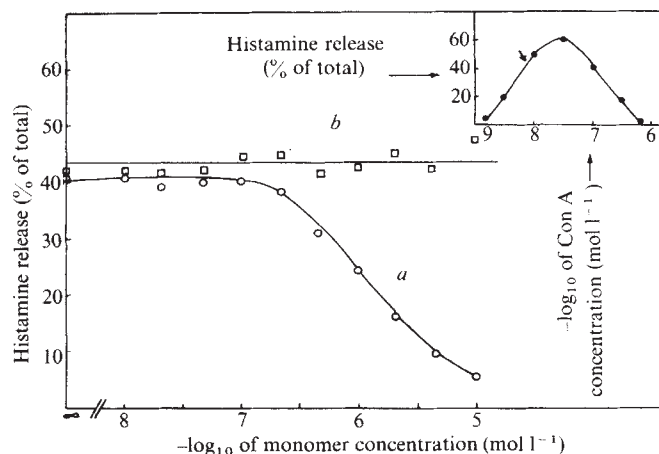


Fig. 1 Histamine release from the basophils of a nonallergic individual (A.A.). Cells were obtained by sedimentation of whole blood. The reaction was carried out at 37° C for 50 min in a Tris-buffered solution (pH 7.4) containing 0.03% human albumin, 1×10^{-3} M Ca²⁺, 1×10^{-3} M Mg²⁺ and 2×10^6 leukocytes per ml corresponding to 20–30 ng histamine base per ml (ref. 24). Supernatants were assayed for histamine by a spectrofluorometric technique^{25,26}. Blanks were less than 5% of the total histamine content. In estimating the molar concentrations, 71,000 daltons¹⁸ was assumed for con A, and 50,000 daltons⁵ for the monomer preparations. *Inset*, histamine release induced by con A. The arrow on the dose-response curve indicates the concentration of con A (6×10^{-9} M) used to obtain the data in the other two curves. ○, The effect of increasing concentrations of monomer Fc specific anti-IgE (abscissa) on the release of a fixed amount of con A (6×10^{-9} M). □, The effect of increasing concentrations of monomer anti-Fab (abscissa) upon the release of a fixed amount of con A (6×10^{-9} M).

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Possible role for the Fc receptor on B lymphocytes

B LYMPHOCYTES¹, polymorphonuclear cells², mast cells³ and cells of the macrophage-monocyte² series possess surface receptors for the Fc portion of the immunoglobulin molecule. In some cases the functions of this receptor is clearly understood but this is not true for B lymphocytes. It has been suggested that the Fc receptor on B cells plays a role in the localisation of antigen in the form of antigen-antibody complexes⁴. We propose an alternative hypothesis that the Fc receptor on B cells is a membrane receptor (termed the prореceptor) for the immunoglobulin which functions as antigen receptor on these cells and present some evidence compatible with this hypothesis.

The plasma cell tumour MOPC 21 (P3) which secretes IgG1 kappa molecules in tissue culture⁵ has surface immunoglobulins but lacks Fc receptors⁶. Mutants which secrete altered immunoglobulin molecules or no immunoglobulin can be obtained^{7,8}, and the nonsecretors lack surface immunoglobulin⁶. According to our hypothesis the apparent absence of Fc receptors on P3 cells would be due to the occupation of Fc receptors by surface immunoglobulin. If this was the case it would be expected that nonsecretors lacking surface immunoglobulin would show the presence of Fc receptors. The results of our investigations on several cell lines are summarised in Table 1.

This shows that in accordance with the hypothesis there is in general an inverse correlation between the presence of surface immunoglobulin and Fc receptors on the cell lines. However there is a variation in the intensity of the Fc receptor reaction on the different nonsecretor lines with X60

Table 1 Properties of the cell lines

Cell line	Secreted molecules	Fc receptors	s Ig
P3	IgG	—	++
X27	IgG	—	++
X63	IgG	—	++
IF2	IgG with C _H 1 region deleted	+	++
IF1	IgG with C _H 3 region deleted	++	+
D1-20	Nonsecretor	+	—
Pr4	Nonsecretor	+	—
289-16	Nonsecretor	+++	—
X60	Nonsecretor	—	—

Surface immunoglobulin was detected with a fluorescein-conjugated IgG fraction of a sheep anti-mouse Fab and a rabbit anti-mouse IgG antiserum⁶. Fc receptors were detected by a rosette technique using bovine erythrocytes coated with mouse antibody as indicator cells^{6,13}. Tumour cells were grown in RPMI 1640 medium (Flow Laboratories, Scotland) containing 15% foetal calf serum. X27 was a cloned cell line derived from a P3 culture. X63, IF1, Pr4 and 289-16 were cloned from X27 cells, while X60, D1-20 and IF2 were isolated from a P3 culture.

Because of variability from test to test, a four point scale is used to describe the Fc receptor reaction on different lines. +++ denotes in general a reaction on greater than 50% of live cells from a culture; ++ a reaction on 10–50%; + a reaction on up to 10% of cells while negative is not significantly above controls with unsensitised indicator cells. The surface immunoglobulin staining on the secretor cells is weaker than on B lymphocytes and a two point scale is used to describe the intensity of staining.

showing no reaction. The mutations leading to nonsecretion are unlikely to be simple and could also affect other aspects of cell function. The variation in the Fc receptor reaction could be a consequence of this. The cell lines IF1 and IF2 are more relevant as the mutations are likely to be more restricted to the structure of the immunoglobulin molecules. The weaker reaction on IF1 for surface immunoglobulin is accompanied by the appearance of Fc receptors. IF2 shows a weak Fc receptor reaction but there is no detectable decrease in surface immunoglobulin. This indicates that the C_H3 region is important in the binding of IgG to the Fc receptors. This was confirmed by using purified protein to inhibit Fc rosette formation. In the experimental conditions used, the wild type and IF2 protein are inhibitory while IF1 protein is not (Table 2).

The relation between surface immunoglobulin and Fc receptors found on the cell lines is consistent with our hypothesis provided that the surface immunoglobulin on secretor cells is not secreted immunoglobulin passively absorbed from the culture medium. Experiments where 289-16 cells were incubated with P3 IgG and then washed and stained with the rabbit anti-globulin showed that immunoglobulin could be passively absorbed. Taking advantage of the observation that rabbit IgG is recognised by mouse Fc receptors⁴ we find that the presence of 10% rabbit serum can prevent absorption of mouse IgG from medium containing 50 mg ml⁻¹ IgG. In contrast, the surface immunoglobulin on secretor cells is not lost from cells grown in 10% rabbit serum for up to 7 d. This shows that the surface immunoglobulin on the secretor cells is not passively absorbed.

The majority of B lymphocytes in mouse possess monomeric IgM receptors^{9–11} but secreted IgM is found to only weakly inhibit Fc rosettes on B cells^{1,12}. One explanation is that receptor IgM may have a higher affinity for Fc receptors than secreted IgM. This possibility is being investigated at present.

Reports in the literature indicate a large number of murine leukaemias of non-T-cell origin which possess Fc

Table 2 Inhibition of Fc rosettes on 289-16 cells with P3, IF1, and IF2 proteins

Inhibitor	% reacting cells ± s.e.	% inhibition
Nil	24.0 ± 4.2	—
P3(580 µg ml ⁻¹)	5.8 ± 1.7	75.5 P < 0.05
IF1(580 µg ml ⁻¹)	5.8 ± 1.7	1.7 (P not significant)
IF2(580 µg ml ⁻¹)	6.4 ± 3.5	73.4 P < 0.05

receptors but no surface immunoglobulin^{6,14}. A light chain secreting variant of a plasmacytoma is also found to be Fc receptor positive^{1,12}. In patients with X-linked agammaglobulinaemia cells are found which lack surface immunoglobulin and possess Fc receptors^{15,16}. We suggest that these may also be cases where the Fc receptors are exposed in the absence of immunoglobulin.

Finally we would like to suggest that in view of the observations that antigen-antibody complexes can induce nonspecific transformation in lymphocytes¹⁷ it is possible that the specific activation of B cells by antigen is mediated through proreceptors.

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Con A differentiates among grass pollens by binding specifically to wall glycoproteins and carbohydrates

AIRBORNE grass pollens are a major cause of hayfever. The allergens involved are mostly proteins and glycoproteins^{1,2}, which are stored in pollen walls³ and rapidly released on moistening and which seem to play a recognition role in pollen/stigma interactions⁴. The capacity of certain plant lectins to bind specific carbohydrates and glycoproteins⁵ and form precipitates with them in double diffusion tests^{6,7} indicates a means of investigating these substances in pollen. We report here how concanavalin A (con A), the lectin from jack beans, *Canavalia ensiformis*, differentiates among grasses by specifically precipitating wall-held materials from

pollens mainly of nonfestucoid species. Furthermore, pollen of corn (*Zea mays*) and its closest relatives is easily distinguishable from that of other grasses by its characteristic reactions with con A.

We have used double diffusion tests to screen grass pollen from 42 species (33 genera, including many known to be important sources of allergens) for materials precipitating with con A. Extracts from 9/42 species have given clear precipitation lines against con A (Table 1). The contrast between festucoids (1/23 reacting clearly) and nonfestucoids (8/19) is marked. In addition, the reacting andropogonoids and *Cynodon* give denser precipitates than the rest, while Maydeae alone (*Zea*, *Euchlaena*, *Tripsacum*) produce two definite bands against con A (Fig. 1c). The relatively faint precipitates with *Festuca*, *Phragmites*, *Stipa* and *Echinochloa* are consistently reproducible (Fig. 1a and b). When diffusates of the positively-reacting pollens of *Cynodon*, *Sorghum*, *Zea* and *Festuca* and of the negatively-react-

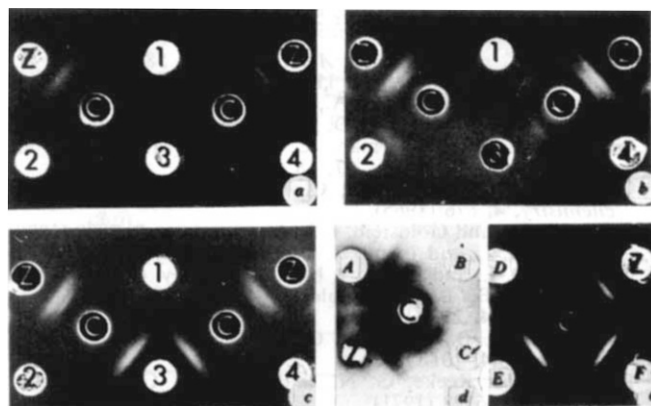


Fig. 1 Double diffusion tests of grass pollen extracts (outer wells) against con A (inner wells). Extracts prepared as in Table 1. *a*, Festucoids: 1, *Lolium multiflorum*; 2, *Festuca rubra*; 3, *Poa annua*; 4, *Bromus inermis*. *b*, Non-festucoids: 1, *Phragmites australis*; 2, *Cynodon dactylon*; 3, *Stipa falcata*; 4, *Danthonia ariantha*. *c*, Nonfestucoids: 1, *Sorghum halapense*; 2, *Coix lacrymajohi*; 3, *Euchlaena mexicana*; 4, *Setaria geniculata*. Arrows indicate faint precipitation lines. *d*, Reactions of con A to various *Zea* diffusate fractions. After Sephadex G25 gel filtration, *A*, a high molecular weight fraction (>5,000 daltons) shows two clear bands; *B*, a low molecular weight fraction (<5,000 daltons) shows no precipitation band; *C*, a fraction from ultrafiltration with a molecular weight of >10,000 daltons, showing precipitation corresponding to inner band of the pair present with whole extract. *e*, Reactions of con A to *Zea* extracts after proteolytic digestion or heat treatment. *D*, after treatment with insolubilised pronase; *E*, after treatment with papain; *F*, after incubation for 1 min at 100°C. *a*, *b*, *c*, and *e*, photographed by dark field illumination of unstained gels; *d*, dried gel stained with Coomassie Blue. *Z*, *Zea mays*; *C*, con A.

ing *Lolium perenne* were concentrated by ultrafiltration and partially purified by Sephadex G25 chromatography, *Lolium* continued to give a negative result, *Cynodon* now gave no reaction while the two andropogonoids and *Festuca* precipitated con A as before. The *Cynodon* reaction probably involves carbohydrate diffusates rather than glycoproteins, and other faintly-precipitating species (where shortage of pollen has precluded more detailed tests) may fall into the same category. A high molecular weight *Zea* fraction (>10,000 daltons) shows two intense precipitation bands (Fig. 1d), there being only slight precipitation in a lower molecular weight fraction probably containing carbohydrates.

The two lines against *Zea* extracts are probably con A-bound glycoproteins, as one is eliminated and the other rendered fainter by previous treatment with pronase (Fig. 1e). Preliminary experiments show that the con A-bound substances are antigenic and correspond in electrophoretic

mobility with two of the five antigens we have detected with high titre rabbit antiserum to *Zea* pollen diffusate⁸. Con A continues to precipitate extracts after incubation for 1 or 20 min at 100° C (Fig. 1e) and it is interesting that similar heat stability is also characteristic of some of the 'I' antigens, considered to be the main allergens of other grass pollens^{1,9}.

When pollen is sprinkled on con A gels, both *Zea* and *Euchlaena* grains show radially-aligned wisps of precipitate within 2 min (Fig. 2a). After 3 h, intense deposits of precipitate were evident (Fig. 2b). These reactions do not occur in control preparations without con A (Fig. 2d), and when other pollens are mixed with *Zea* as controls of specificity (Fig. 2c) conspicuous precipitates form only around the *Zea* grains even after 24 h incubation. We have also modified immunofluorescence procedures for pollen antigen location^{10,11}, to detect sites of con A binding to glycoproteins or carbohydrates in freeze-sectioned *Zea* and *Tripsacum* grains. Intensely-fluorescing precipitins appeared only in pollen wall sites (Fig. 3a), on the surface of the outer exine and in the inner cellulosic intine layer, and were absent from appropriate controls (Fig. 3b). The diffusible antigens of *Phalaris tuberosa* pollen have been located in these same sites³, and show characteristic release kinetics. Immediately grass pollen grains are moistened, proteins and glycoproteins stored in cavities of the exine are released through surface micropores¹. Only after about 3 min do those of the inner intine begin to be released, through the single pore or germinal aperture. While *Zea* and *Euchlaena* pollen precipitates con A within 2 min of moistening, the considerable increase in precipitation by 3 h indicates the involvement of both wall fractions. The immunofluorescence experiment seems to confirm this.

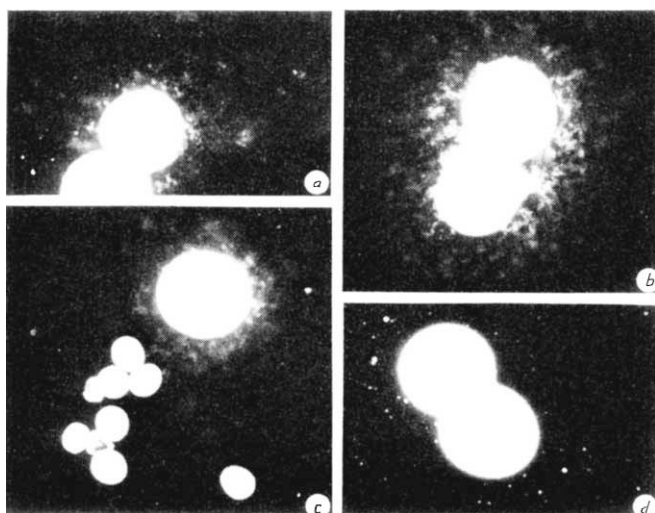


Fig. 2 *Zea* pollen sprinkled on 0.5% agarose gels containing 2 mg ml⁻¹ con A and 0.2 M NaCl, viewed by dark field microscopy: a, After 2 min showing precipitate around grain; b, after 3 h showing dense radiating precipitates; c, mixture of *Zea* and *Lolium* pollen after 3 h with precipitation confined to the large *Zea* grains and absent from *Lolium* grains (in sharp focus); d, control with *Zea* grains sprinkled on gels without con A. ($\times 270$).

It is not widely known that allergic persons often show taxonomically-patterned responses to festucoid, chloridoid, panicoid and andropogonoid grasses (refs 12, 13 and unpublished data). Methods have not yet been devised for distinguishing airborne grass pollens so it remains unclear how far this phenomenon results merely from factors affecting atmospheric availability of pollen for sensitisation. Our observations with con A, however, are consistent with the possibility that it directly reflects taxonomically-ordered variation in the allergenic components of pollen grains, as

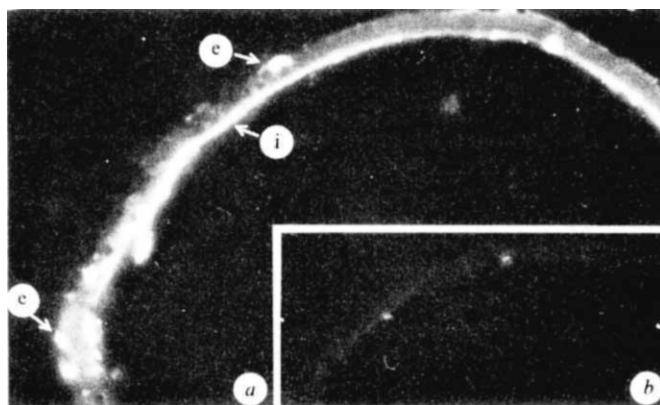


Fig. 3 Immunofluorescence localisation of con A-binding substances in freeze-sectioned *Zea* pollen, fluorescence image. a, Section treated with con A (40 min), saline rinse (15 min), rabbit anti-con A serum (40 min), saline rinse (15 min), FITC-labelled anti-rabbit γ -globulin serum (40 min); fluorescing precipitins present in intine (i) and surface exine cavities (e) but absent from protoplast; b, control section treated with con A, saline rinse, normal rabbit serum, saline rinse and FITC-labelled anti-rabbit γ -globulin serum, no fluorescing precipitins evident. ($\times 900$)

are the findings of Augustin¹³ and Marsh *et al.*⁹ who, while working mostly with festucoids, have found a few non-festucoids to be immunologically very distinct. Such taxonomic predictability needs pursuing in detail, as it has medical potential for detecting cross reactions among allergenic pollens and for efficient selection of appropriate extracts or allergoids¹⁴ for desensitisation therapy. It also points to the need in atmospheric pollen counts to try and identify the grasses, at least to major group level, and Fig. 2c indicates how further work with other lectins may help.

Many of the materials released when pollen is moistened, including proteins, glycoproteins and carbohydrates, function in early germination processes on the stigma. In *Phalaris tuberosa*, rapidly-released antigens bind to the stigma surface³. They seem to act in a variety of ways as pollen/stigma recognition substances¹, being concerned in regulating the breeding system. It is therefore intriguing that pollens of cultivated corn (*Zea mays*) and species directly involved in its evolution (*Euchlaena*, *Tripsacum*)¹⁵ produce an identical and unique reaction with con A.

Table 1 List of grass pollen extracts tested with con A by double diffusion in agarose

Festucoids

Agropyron repens, *Agrostis tenuis*, *Anthoxanthum odoratum*, *Avena barbata*, *Bromus carinatus*, *B. inermis*, *B. rigidus*, *Dactylis glomerata*, *Elymus condensatus*, *E. triticoides*, *Festuca rubra*, *Holcus lanatus*, *Hordeum vulgare*, *Lolium multiflorum*, *L. perenne*, *L. temulentum*, *Phalaris arundinacea*, *Ph. minor*, *Ph. tuberosa*, *Poa annua*, *P. pratensis*, *P. scabrella*, *Secale cereale*

Chloridoids

Bouteloua sp., *Chloris truncata*, *Cynodon dactylon*, *Distichlis spicata*, *Sporobolus aeroides*

Arundinoid—Danthonioids

Danthonia eriantha, *Phragmites australis*, *Stipa falcata*

Panicoids

Echinochloa crus-galli, *Paspalum dilatatum*, *Pennisetum villosum*, *Setaria geniculata*

Andropogonoids

(1) Andropogoneae: *Saccharum officinarum*, *Sorghum halapense*, *Themeda australis*

(2) Maydeae: *Coix lacrymajobi*, *Euchlaena mexicana*, *Tripsacum floridanum*, *Zea mays*

Those showing precipitation appear in **bold face**. 0.2 g ml⁻¹ dry pollen (obtained from Greer Laboratories, Lenoir, North Carolina; Commonwealth Serum Laboratories, Melbourne, Australia, and collected by us) extracted in 0.2 M NaCl in 0.05 M tris buffer, pH 7.5 for 20 min at room temperature. Con A (Pharmacia) used at 2 or 5 mg ml⁻¹ in same buffered saline.

In *Silene* the pollen wall proteins bind to the stigma surface pellicle, a protein overlay found in most if not all angiosperms and which is the probable site of recognition reactions determining acceptance or rejection¹⁶. An interpretation of the situation in *Zea* and its closest relatives open to experimental testing (comparable with mammalian sperm/egg recognition¹⁷) is that the stigmatic surface receptors may possess specific determinants similar to those of con A.

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3 mM BaCl₂. This 'modified' Krebs solution was used to inhibit Ca²⁺, Mg²⁺ dependent ATPase activity, and it contained Ba²⁺ to provide the divalent cation requirement for ACh release⁶. The release of ACh was evoked either by stimulating the preganglionic sympathetic trunk (20 Hz, 0.3 ms, 5.5 V) or by raising the KCl concentration in the perfusion medium to 50 mM (in which case the NaCl concentration was reduced to 50 mM). The effluent from the ganglion was collected in 5 min aliquots and each sample was assayed for ATP and for ACh. ATP was assayed using a modification of the luciferase method⁷ that permitted the determination of as little as 4 pmol ATP; ACh was measured by bioassay on the cat blood pressure⁸. In some experiments adenosine monophosphate (AMP) or adenosine diphosphate (ADP) was measured instead of ATP; they were measured by the luciferase method after being converted to ATP enzymatically⁹. The conversion of AMP or ADP to ATP was checked by thin-layer chromatography¹⁰ using ¹⁴C labelled standards.

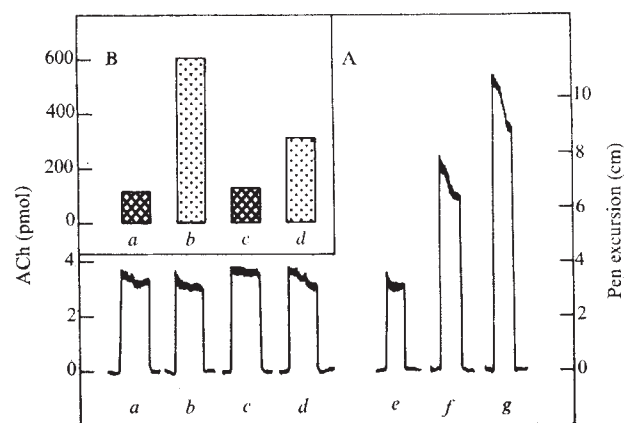


Fig. 1 A, Chart record of light emitted in the ATP assay of perfusate collected from the ganglion during a, rest; b, stimulation by high K⁺; c, rest; d, preganglionic nerve stimulation. e, Control sample of 'modified' Krebs solution with no added ATP; f, 7.5 pmol ATP added to 'modified' Krebs medium; g, 12.5 pmol ATP added to 'modified' Krebs medium. ATP was assayed as follows. A plastic vial containing 1.5 ml of the sample to be assayed was placed in a light-tight housing system which was connected to a photomultiplier (American Instrument Co., USA) and to a Texas Rectiriter pen recorder. The reaction was initiated by injecting 1.0 ml of firefly lantern extract (Sigma, 5 mg ml⁻¹ dissolved in 0.15 M Na₃AsO₄ buffer, pH 7.5, containing 0.1 M MgSO₄) into the vial through a rubber seal on the top of the housing system. B, The amount (pmol) of ACh contained in the same samples measured on the blood pressure of the eviscerated cat⁸. Before measuring the ACh content of the perfusion medium, barium ions were removed by the addition of 1/10 volume of 0.15 M Na₂SO₄; the precipitated BaSO₄ was removed by centrifugation and the supernatant was used for bioassay.

Absence of adenine nucleotide release from autonomic ganglion

SYNAPTIC vesicles isolated from Torpedo electroplaques contain ATP as well as acetylcholine (ACh)^{1,2}. Silinsky and Hubbard³ showed that ATP is released from the rat hemidiaphragm bathed in a hyperosmotic medium and they suggested that the motor nerve terminal releases ACh and ATP simultaneously. Here we report tests for the release of ATP and ACh from the cat superior cervical ganglion; we demonstrated that ACh, but not ATP, is released during stimulation.

The superior cervical ganglion of the cat was perfused^{4,5} either with 'normal' Krebs solution (composition in mM: NaCl, 120; KCl, 4.6; CaCl₂, 2.4; NaHCO₃, 25; KH₂PO₄, 1.2; MgSO₄·7H₂O, 1.2; glucose, 9.9; neostigmine bromide, 0.03) or with a Krebs solution that did not contain CaCl₂ or MgSO₄ but contained 1 mM EDTA and

Figure 1 illustrates the result of a typical experiment which measured the release of ACh and ATP by nerve stimulation or by raised K⁺ during perfusion with 'modified' Krebs solution. Although ACh was released by perfusion with high K⁺ or by nerve stimulation, ATP could not be detected during the stimulation or rest period. Six other experiments confirmed this result. At the end of each experiment, a known concentration of ATP (60 pmol ml⁻¹ in 'modified' Krebs solution) was perfused through the ganglion to determine the extent of breakdown. This usually was 10–25%. In contrast, when normal Krebs plus ATP or Krebs medium containing 0.1 mM Ca²⁺ plus ATP (no Mg²⁺) was perfused through the ganglion, none of the ATP survived degradation. Whittaker *et al.*¹ found that the mean ACh:ATP molar ratio was 11:1 in synaptic vesicles from Torpedo electroplaques. If this ratio were the same

in transmitter stores of cat ganglion, and if all of the ATP were released during stimulation, then in our conditions, in which 150–650 pmol of ACh was released, 14–59 pmol of ATP should have been released; this quantity of ATP would have been readily detected under the conditions of our experiments. It is interesting that ATP release was detected immediately following a change of perfusion medium from a normal Krebs to a 'modified' Krebs solution. At this time a transient depolarisation of the postganglionic fibres occurred, causing a contraction of the nictitating membrane and about 15 pmol of ATP was released; no ACh was released during this period. We did not determine the origin of this ATP.

Other experiments were designed to test the release of ADP or AMP when perfusion was with normal Krebs medium and ACh release was evoked by nerve stimulation. In all experiments, ACh was released during nerve stimulation, but no ADP or AMP release could be detected. At the end of each experiment, ADP or AMP of known concentration was perfused through the ganglion, collected and analysed to determine the extent of breakdown. The ADP and AMP were degraded by 25% and 10% respectively.

In conclusion, we were unable to reproduce the results of Silinsky and Hubbard³ using a different mammalian preparation. It is possible that the ATP detected in their experiments originated from the muscle rather than from the nerve terminal, as suggested by other workers^{11–13}. The perfusion of the superior cervical ganglion avoided any artefacts arising from the presence of muscle, and allowed the collection of more ACh than could be collected from the stimulated rat phrenic nerve diaphragm preparation.

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Pattern of remyelination in the CNS

It has been suggested that reduction of internodal length may be a feature of remyelinated central nerve fibres as it is of peripheral nerve fibres¹. As the technique for measuring internodal length of nerve fibres in the central nervous system (CNS) is tedious and requires a high degree of skill and patience from the investigator², it is unlikely that this method can be applied to routine neuropathological investigations. Recent ultrastructural studies on remyelination in the mouse, following primary demyelination induced by feeding cuprizone (Bis - cyclohexanone - oxalaldehyde) have shown that measurements of the relationship between myelin sheath thickness and axon diameter provides an easier method of identifying remyelinated axons.

Male weanling albino mice of the ICI strain were fed on a powdered mouse diet containing 0.6% cuprizone. Two mice were killed after 6 weeks to assess the extent of demyelination in the superior cerebellar peduncle (SCP) (ref. 3) (Table 1). As both did not show complete demyelination the remaining nine animals were left on the cuprizone-containing diet for a further 2 weeks. At this time two animals were killed and examined for demyelination. Nearly all fibres in the SCP were demyelinated (Table 1), so the remaining seven animals were placed on a normal diet (previous experiments having shown that under these circumstances remyelination readily takes place^{4,5}). Mice were then killed at the times indicated in Table 1 and remyelination assessed. Normal mice of two different ages were also killed and examined in the same way as the experimental animals. All animals were perfused with 4% glutaraldehyde in phosphate buffer and blocks were taken

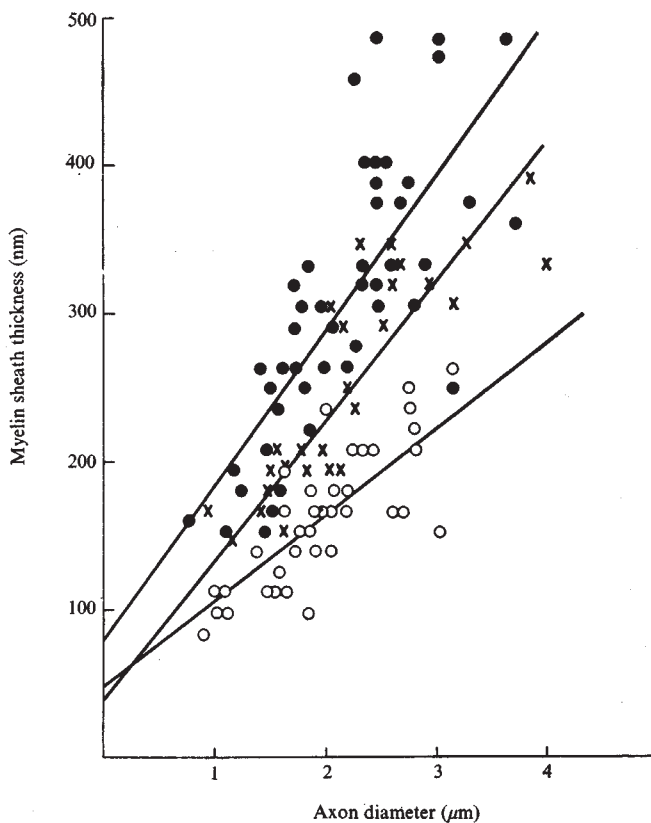


Fig. 1 Relationship between myelin sheath thickness and axon diameter in the superior cerebellar peduncle in a young mouse (x), an old mouse (●), and a mouse 6 months after this tract was demyelinated (○). Animals marked with a dagger in Table 1.

Table 1 Remyelination in superior cerebellar peduncle

	% myelinated fibres	Mean d/D ratio	SD	Regression equation* $Y = \bar{y} + byx(x - \bar{x})$	Correlation coefficient
Old normal (9 month)	100 100	0.78 0.76	± 0.03 ± 0.03	$Y = 22.51 + 1.56(x - 15.99)$ $Y = 24.47 + 1.48(x - 15.53)$	0.74† 0.85
Young normal (2 month)	100 100	0.81 0.81	± 0.02 ± 0.03	$Y = 15.61 + 0.95(x - 14.03)$ $Y = 16.94 + 0.93(x - 15.12)$	0.83 0.89†
6 weeks cuprizone	98 3				
8 weeks cuprizone	15 4				
8 weeks cuprizone 6 weeks recovery	90 79	0.92 0.91	± 0.03 ± 0.03	$Y = 7.33 + 0.24(x - 16.65)$ $Y = 7.29 + 0.09(x - 16.37)$	0.50 0.19
8 weeks cuprizone 12 weeks recovery	56 90 88	0.92 0.90 0.90	± 0.03 ± 0.02 ± 0.02	$Y = 6.61 + 0.28(x - 15.26)$ $Y = 8.74 + 0.55(x - 15.39)$ $Y = 7.22 + 0.32(x - 14.42)$	0.46 0.74 0.56
8 weeks cuprizone 24 weeks recovery	88 98	0.89 0.86	± 0.03 ± 0.02	$Y = 8.87 + 0.34(x - 15.30)$ $Y = 12.13 + 0.58(x - 14.78)$	0.48 0.73†

* In regression equation: $\bar{y}$ = mean myelin sheath thickness; $\bar{x}$ = mean axon diameter; byx = slope of linear regression of myelin sheath thickness on axon diameter; x and y were measured in mm on electron micrographs at 7,200 magnification; y = actual measurement $\times 10$.

† Indicates the three animals depicted graphically in Fig. 1.

d = diameter of axon; D = diameter of fibre.

which contained the SCP cut in transverse section at the point where it passes under the middle cerebellar peduncle^{3,4}. Thin sections were prepared for electron microscopy and six electron micrographs were taken at random of the SCP at a magnification of 1,800. These were enlarged photographically a further four times and the following parameters measured: percentage of myelinated fibres, myelin sheath thickness and axon diameter. These were determined for a representative sample of fibres by methods used previously^{3,4}. Table 1 gives values for the percentage of myelinated fibres in the SCP, the d/D ratio (diameter of axon/diameter of fibre)—an index of myelin sheath thickness—the slope of the linear regression of myelin sheath thickness on axon diameter, and the correlation coefficient of myelin sheath thickness and axon diameter. An analysis of the significances of differences between the regression lines was carried out⁶.

Nearly all axons are remyelinated during the recovery period and with time the myelin sheath increases in thickness (Table 1). But even after six months recovery, myelin sheath thickness has not returned to its normal relationship with axon diameter (Table 1, Fig. 1). There is a significant difference between the index of myelin thickness for the 6 month remyelinated animals and the 2-month-old control animals. Taking into account that the 6-month-recovery animals are 10 months old, it can be seen that the remyelinated axons have much thinner myelin sheaths than would be expected for animals of their age. In addition the slopes of the regression lines for the remyelinated animals are significantly different (at the 0.01 level; sample size 32–47 observations) from either young or old control animals. It seems therefore that although myelin sheath thickness does increase with time in remyelinated animals, it does not return to its normal relationship with axon diameter for a considerable length of time. It is likely, therefore, that the presence of an axon within a myelin sheath which is thinner than would be expected for the diameter of the contained axon, represents a remyelinated fibre. Such an observation will have little significance when applied to a single fibre, but if it can be shown that the axon diameter–myelin sheath thickness relationship for a population of fibres is abnormal, or that two populations of fibres coexist in a single tract then it can be reasonably assumed that remyelination has taken place on the axons possessing thinner than normal myelin sheaths. This experiment supports the many observers who

have stated that axons within thinner than normal myelin sheaths represent remyelinated axons^{7–12} and suggests a simple method for determining the extent of remyelination in man.

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Effect of intracellular lithium on snail neurones

It is well established that lithium salts are effective in the treatment of manic depressive illness^{1,2}, but the way they act is little understood. Some time ago, as part of a study on the electrogenic sodium pump, it was noticed that high levels

of intracellular Li^+ or Na^+ seemed to increase the potassium conductance of the snail nerve cell membrane^{3,4}. Because of the greatly increased interest in Li^+ itself, we have now confirmed the original observations, and have shown that detectable effects occur at quite low levels of intracellular Li^+ . Such an increased membrane conductance to potassium would tend to decrease the membrane excitability and hence stabilise the cell. If a similar response to intracellular Li^+ occurs in man, it could be the basis for its therapeutic effects.

We performed our experiments on the largest nerve cell at the rear of the right pallial ganglion of the common snail *Helix aspersa*. The brain was dissected from dormant specimens and pinned in a chamber where it was bathed in continuously flowing snail Ringer (KCl , 4 mM; NaCl , 80 mM; CaCl_2 , 7 mM; MgCl_2 , 5 mM; Tris-maleate (pH 7.5), 20 mM) at room temperature (14–21°C). The bathing solution could be quickly changed to Ringer containing various concentrations of potassium or to normal Ringer with 10^{-4} M ouabain by means of a multiposition tap. The cell was impaled with one double and two single-barrelled microelectrodes. This allowed for the recording of membrane potential, the determination of input resistance by injected current pulses, and the interbarrel injection of LiCl , NaCl or KCl into the cell. In this last technique, current is passed between two intracellular electrodes, ejecting cations from one electrode and anions from the other.

Cell diameter was measured and cell volume calculated assuming a spherical cell. The amplitude and length of the interbarrel injection current was recorded and thus the amount of salt injected could be estimated. (For a more detailed description of these methods, see Thomas⁵.)

The effect of a relatively large injection of LiCl is shown in Fig. 1. With the intracellular Li^+ concentration raised to a calculated level of 29 mM, the membrane potential was increased by about 3 mV, and the cell's spontaneous action potentials ceased. (Larger effects were seen with larger injections of LiCl , but in this experiment injection was stopped when the cell ceased firing to ensure reasonably rapid recovery.) There was a simultaneous one-third reduction in input resistance, as is shown by the decrease in the size of the hyperpolarisations resulting from the current pulses. Following the injection, both input resistance and membrane potential gradually returned to their resting values and spontaneous action potentials returned. The lowered resistance after LiCl injection recovered approximately exponentially. The time constant for the recovery shown in Fig. 1 was 40 min. Similar effects were seen in 29 cells, although in several cases the cell did not recover after the injection.

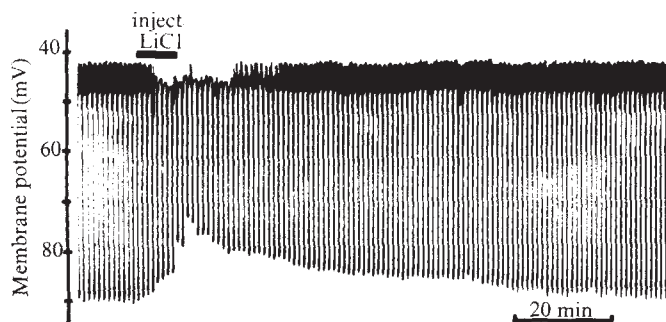


Fig. 1 Pen recording from an experiment to show the effects of injected LiCl on the input resistance of a spontaneously active snail neurone. The slow time response of the recorder has reduced the apparent size of the action potentials to only a few millivolts. Every minute a 5 nA hyperpolarising current was passed across the cell membrane for 10 s to measure the input resistance. The solid bar indicates the 8 min period during which an interbarrel injection current of 25 nA was passed. The cell diameter was 0.2 mm, so that this injection would be expected to raise the intracellular Li concentration by about 29 mM.

We also tested the effects of large injections of KCl and NaCl . An injection of KCl which would be expected to raise the internal K^+ concentration by about 45 mM caused only a slow depolarisation of a few millivolts with no change in input resistance. The injection of NaCl produced a more complex and variable result. Small injections of NaCl consistently caused an immediate ouabain-sensitive hyperpolarisation (as expected from the consequent stimulation of the electrogenic Na pump⁴) with no appreciable change in input resistance. Large injections of NaCl (giving a concentration greater than 20 mM), however, often produced both a considerably greater hyperpolarisation, which was little affected by ouabain, and a noticeable decrease in input resistance.

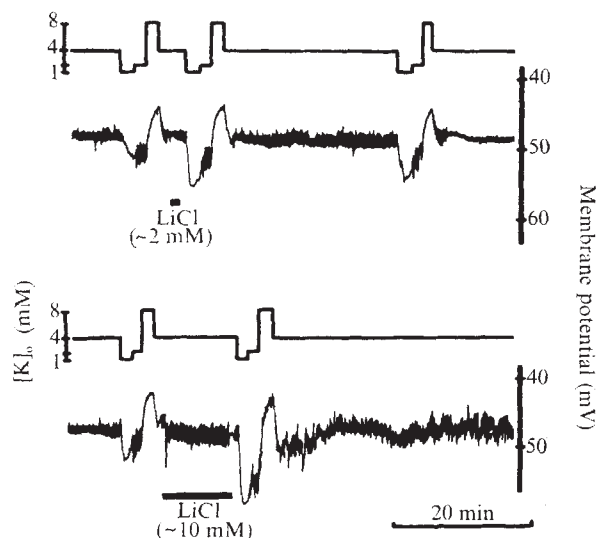


Fig. 2 The effect of injected LiCl on the sensitivity of a snail neurone to changes in external potassium. Two segments from a continuous record showing the effects on the membrane potential of brief exposures to 1, 2 and 8 mM external potassium. The normal external potassium concentration ($[\text{K}]_o$) was 4 mM. LiCl was iontophoretically injected where indicated by the solid bars, giving estimated internal concentrations of 2 and 10 mM.

Another way of describing the experiment shown in Fig. 1 is to say that the cell was being held, alternately, at two membrane potentials; one, the resting level at the top of the current pulses (~ 45 mV) and the other a more hyperpolarised level at the bottom of the pulses (~ 90 mV). Following a LiCl injection, then, the resting cell is seen to hyperpolarise while a cell held at a hyperpolarised potential depolarises, thus indicating the existence of an equilibrium potential for the process. The large injections of Cl^- which accompany these Li^+ injections would cause the chloride equilibrium potential to be less negative than the resting potential. Potassium is the only ion whose equilibrium potential would be in the range to produce these effects, although some involvement of other ions cannot be ruled out.

Figure 2 shows an experiment where the external K^+ concentration ($[\text{K}]_o$) was changed before and after two injections of LiCl . Although quite large injections are necessary to produce the marked changes in input resistance seen in Fig. 1, measurable changes in the relative potassium permeability (P_K) can be produced by much smaller injections. Following both LiCl injections in Fig. 2 the membrane potential becomes more responsive to a change in external K^+ . As the injected Li^+ leaves the cell, this increased sensitivity declines gradually to the resting level.

Estimates in the change in P_K before and after LiCl injections, assuming no change in other ion permeabilities, were obtained from linear regression fits to $[\text{K}]_o$ against $\exp(FV/RT)$

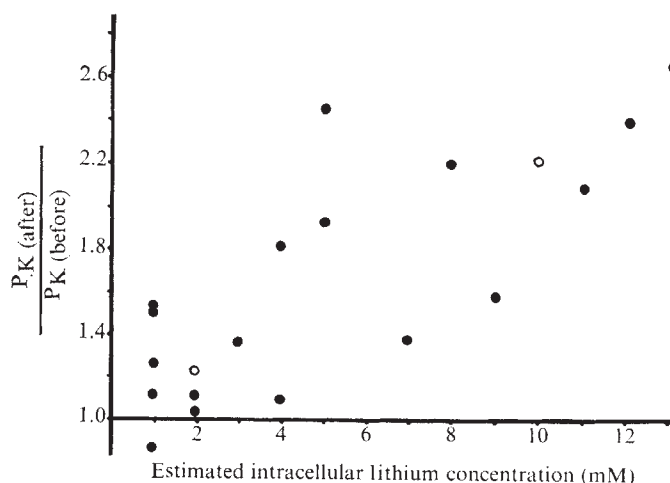


Fig. 3 Graph of the changes in the relative potassium permeabilities (P_K) of snail neurones as a function of estimated intracellular Li concentration. Relative P_K s are calculated from plots of $[K]_o$ against $\exp(FV/RT)$. Results from 20 injections in seven cells; ○, points calculated from the data shown in Fig. 2.

plots (ref. 6) of 20 LiCl injections in seven cells. Figure 3 shows these changes in P_K as a function of the estimated intracellular Li^+ concentration. Although there is considerable scatter, the trend of a rising P_K following successively larger LiCl injections can be clearly seen. Thus intracellular Li^+ injections giving concentrations of a few mM cause measurable increases in P_K and injections of greater than about 8 mM more than double the resting P_K .

The extracellular concentration of Li^+ that is effective in the treatment of manic depressive illness is around 1 mM (refs 1 and 2). It seems possible that at least some neurones in the human brain will tend to concentrate Li^+ intracellularly, as passive distribution alone would give an internal level of about 10 mM in a cell whose resting potential was 60 mV, and the Na-K pump does not extrude Li^+ . Thus, if intracellular Li^+ has the same effect on some human neurones as on snail neurones, its therapeutic effects could arise from the membrane stabilising effects of the increased potassium permeability.

How does Li^+ cause this rise in potassium permeability? Perhaps the most likely mechanism is by an increase in the intracellular ionised Ca^{2+} . Meech⁷ has shown that the injection of $CaCl_2$ into mollusc neurones causes a rise in their potassium permeability, and similar responses have been seen with mammalian motoneurones⁸. There is also evidence that Ca^{2+} is normally extruded from nerve cells by a Na-Ca exchange system⁹, so that a rise in intracellular Na^+ will tend to cause a rise in intracellular Ca^{2+} . If Li^+ can substitute for Na^+ in this system then intracellular Li^+ would tend to cause an increase in intracellular Ca^{2+} . We have some preliminary evidence that intracellular calcium is involved, as we have found that the injection of EGTA partially reverses the change in P_K following a large lithium injection.

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Periodicity in high sulphur proteins from wool

IN a recent publication¹, Elleman, Lindley and Rowlands claimed that there is no evidence to support the view that high-sulphur proteins from wool have a common ancestral protein partly composed of a repeating unit of five amino acids^{2,3}. Their evidence for a repeating decapeptide unit is restricted almost entirely to the region between residues 22 and 64 of protein SCMKB2B. Although the C terminal region of this sequence shows some correlation to the repeating decapeptide unit, it correlates better with a pentapeptide repeating unit as shown previously (ref. 3).

It is informative to compare the 'decapeptide rich' regions of the sequences of the three homologous SCMKB2 proteins⁴ as shown in Fig. 1. Protein B2C contains two decapeptide repeating units while B2B and B2A contain three and four units, respectively. These differences probably resulted from gene elongation due to unequal crossing over of DNA during meiosis. The original decapeptide unit would then have been two adjacent pentapeptide units differing as a result of mutations. This would explain the 'partial symmetry' referred to by Elleman *et al.*¹.

Protein B2A	Protein B2B	Protein B2C
23 P C Q P T	23 C C Q P T	23 C C R S T
C C Q T S	C S Q T S	C S Q T S
C C Q P T	C C Q P T	C C Q P T
S I Q T S	S I Q T S	S I Q T S
C C Q P I	C C Q P I	C C Q P T
S I Q T S	S I Q T S	-----
C C Q P T	C C Q P T	-----
S I Q T S	-----	-----
C C Q P T	-----	-----
C L Q T S	C L Q T S	C L Q T S
G C E ⁷⁵	G C E ⁶⁵	G C E ⁵⁵

Fig. 1 The partial amino acid sequences⁴ of protein SCMK-B2A (residues 23-75), protein SCMK-B2B (residues 23-65) and protein SCMK-B2C (residues 23-55) from the high-sulphur fraction of Lincoln wool, arranged to show maximum homology based on a pentapeptide repeat. The single letter notation for amino acids⁵ has been used.

Lindley and Elleman⁶ showed homology between 14 residues at the C terminal regions of the IIB and B2 proteins. This homology was further extended by aligning a part of the sequence of protein IIIA3 with these regions³, which proves a common origin for all these high-sulphur proteins. Yet Elleman *et al.*¹ derived different homologous decapeptide units for proteins IIIA3 and B2B, and none for the IIB proteins, which they claimed to be random. We found³ a single primordial pentapeptide unit, Cys-Cys-Gln-

Pro-X, which fits the IIIA3 and B2 groups of proteins equally well and, in addition, nearest neighbour statistical analysis shows that the sequences of the IIIB proteins are not random and are derived from the same unit with a probability of the order of 8×10^6 to one.

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Platelet-activating factor, a new mediator of anaphylaxis and immune complex deposition from rabbit and human basophils

AN increase in vascular permeability as a result of the release of vasoactive amines has been implicated as a 'trigger mechanism' of immune complex deposition^{1,2}. Several immunological mechanisms of vasoactive amine release from rabbit platelets have been described³. Experiments have correlated the presence of one of these mechanisms, a complement-independent, leukocyte-dependent reaction, with the vascular deposition of complexes in acute⁴ and chronic⁵ serum sickness of rabbits. We have obtained direct evidence that this mechanism is due to basophils sensitised with IgE (ref. 6). Antigen-induced *in vitro* degranulation of basophils from rabbits immunised with peroxidase yielded a soluble factor (platelet-activating factor (PAF)) which aggregated rabbit platelets in leukocyte-free solutions⁶. Here we describe several characteristics of rabbit PAF and a PAF-like factor obtained from human leukocytes.

Procedures used to obtain PAF from rabbit leukocytes have already been described in detail⁶. Briefly, buffy coat from rabbits immunised with peroxidase was cleared of erythrocytes by sedimentation in phosphate buffered saline (PBS) with 2.5% gelatin followed by osmotic shock, and cleared of platelets by repeated low speed centrifugation. Leukocytes were incubated in Tris-buffered Tyrode's solution (pH 7.4), with 0.25% bovine serum albumin (TTBSA), in the presence of antigen for 15 min at 37° C. Absence of platelets in buffy coat and addition of BSA to the medium as a PAF carrier are prerequisites for recovery of PAF. Cell-free supernatant was obtained by centrifugation, dialysed with 0.01 M phosphate buffer (pH 8.0), chromatographed on a DEAE-Sephadex A 50 anion exchange column, with a gradient of NaCl. PAF activity, measured in an aggregometer by aggregation of rabbit platelets in Tyrode's added with 0.25% gelatin (TG), was eluted with BSA. After dialysis with 0.01 M Tris 0.15 NaCl buffer (pH 7.4), PAF was separated from BSA by precipitation in 80% ethanol. After centrifugation at 60,000g, the supernatant was evaporated to dryness and PAF was resuspended in 80% ethanol. Throughout purification and characterisation of PAF, controls were TTBSA with leukocytes and peroxidase but maintained at 4° C for 15 min, instead of 37° C, or TTBSA without Ca²⁺ and Mg²⁺ used instead of TTBSA in the usual

degranulation method. In a second step, leukocytes used for preparation of control buffers were resuspended in TTBSA at 37° C to release PAF in the presence of peroxidase.

PAF in 80% ethanol was filtered through a Sephadex LH 20 column equilibrated with 80% ethanol at pH 9.5. Markers were ¹²⁵I-labelled glucagon, bacitracin, vitamin B₁₂, ³H-angiotensin and PSP. PAF from rabbit leukocytes (R-PAF) was eluted between vitamin B₁₂ and angiotensin, exhibiting an apparent molecular weight of 1,100.

PAF filtered through LH20 in 80% ethanol was evaporated to dryness and resuspended in distilled water or 80% ethanol. PAF activity disappeared from distilled water-containing tubes in about 24 h, whereas PAF activity remained unchanged in 80% ethanol for several weeks at 4° C. Varying the pH of distilled water between 5 and 10 did not influence the decrease of PAF activity. To explore this phenomenon further, the same experiment was carried out in distilled water, using tubes used for platelet aggregation. Tubes from which PAF activity had disappeared were emptied, rinsed once and filled with TG. Platelet aggregation occurred in these tubes, showing that PAF in distilled water had been adsorbed on the plastic walls of the tubes. No PAF activity was found on the walls of the tubes which had contained PAF in 80% ethanol. This result indicated that further studies of PAF had to be carried out in 80% ethanol.

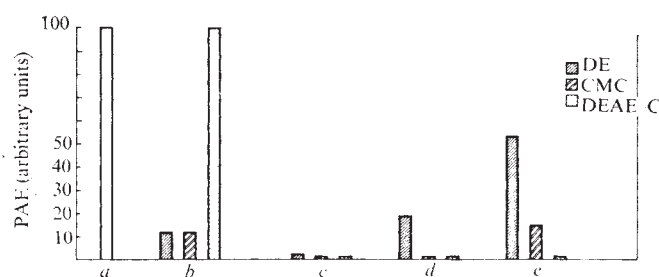


Fig. 1 Adsorption of PAF on charged particles in 80% ethanol. PAF activity was measured by aggregation of 3×10^7 rabbit platelets in 0.3 ml Tyrode's in an aggregometer (Icare, Marseille, France). a, Supernatants before adsorption at pH 6.50; b, Supernatants after adsorption at pH 6.50; c, elution from washed particles at pH 7.00; d, at pH 8.00; e, at pH 9.50.

We attempted to determine the electrostatic charge of PAF by adsorption and elution experiments with negatively charged particles, such as diatomaceous earth (DE) and carboxymethylcellulose (CMC) (Fig. 1). PAF in 80% ethanol at pH 6.5 was incubated with DE or CMC. PAF activity disappeared from the solutions. DE and CMC were washed once with 80% ethanol at pH 6.5, then the pH was gradually raised to pH 11. Elution of PAF was obtained at a pH between 9.2 and 9.7 with a maximum most frequently obtained at pH 9.5. The same experiment was performed in distilled water at pH 6.5 with similar results but very low yield of eluted PAF. These results are compatible with a PAF isoelectric point between pH 9 and 10. Controls for this experiment were: first, binding of arginine by DE or CMC in 80% ethanol, showing that the particles were charged in this medium; second, a lack of binding of PAF and arginine at neutral pH by DEAE-cellulose, a positively charged particle; and third, binding of aspartic acid in 80% ethanol by DEAE-cellulose but not by DE or CMC. Experiments were undertaken to obtain release of PAF from rabbit basophils without antigen-IgE interaction. Overnight slow agitation of leukocyte preparation from immunised or normal rabbits in TTBSA (pH 7.4) at 22° C released small amounts of a PAF-like material (spontaneous release (SR)). In keeping with the basic nature of the molecule, it was postulated that a better release would be obtained at a pH where the molecule has a neutral electrostatic charge. Indeed, SR was

obtained readily at a starting pH of 10.5 (final pH 9.5) within 90 min at 22° C. Through the whole procedure of purification, the molecule obtained by spontaneous release at pH 9.5 showed exactly the same characteristics as PAF actively released at pH 7.4. At pH 9.5, no metabolic activity of the cells was necessary for PAF release—in contrast with the metabolic activity required for release at pH 7.4 (J. Benveniste, unpublished). Human leukocytes were prepared by sedimentation of blood in 2.5% gelatin in PBS, followed by osmotic shock and several washings with TTBSA. Final leukocyte preparations contained 0.2–1% basophils with other leukocytes in normal proportion. Incubation of leukocytes in TTBSA at 22° C gave exactly the same results as in rabbits, that is, small amounts of a PAF-like factor at pH 7.4 and much higher amounts, comparable to those obtained from rabbit leukocytes, at pH 10.6. This human factor bound to BSA, was soluble in 80% ethanol, showed neutral electrostatic charge at pH 9.5 and an apparent molecular weight of 1,100. Further purification and identification of the molecule is under way in our laboratory.

These results indicate that PAF is a basic molecule with a molecular weight of 1,100. Its highly positive charge at physiological pH could explain its tendency to bind to negatively charged surfaces. It is likely that the molecule also possesses hydrophobic sites responsible for its binding to BSA and plastic walls in aqueous but not in 80% ethanol solution. PAF represents a new mediator of anaphylaxis, different from those already described: histamine, SRS-A (ref. 7) and ECF-A (refs 8 and 9). In rabbits, PAF has been clearly identified as of basophil origin: we have obtained the release of PAF by anti-IgE antiserum but not anti-IgG (ref. 6). It has been implicated in immune complex deposition through its aggregating and releasing action on platelets. The identity of the factors obtained from human leukocytes and from rabbit basophils makes the basophil a likely source in the human. Preliminary results in our laboratory show that leukocytes from patients having undergone basophil degranulation during anaphylactic shock do not release PAF, a situation very close to immunised rabbits injected daily with small doses of antigen. The direct evidence of the basophil origin of human PAF remains to be demonstrated, however. If the existence of PAF is firmly established in the human, it could represent, according to the experimental model, the mediator of a mechanism involving IgE-sensitised basophils in the pathogenesis of immune complex deposition. This mechanism would be a link between immediate hypersensitivity and immunological diseases associated with severe structural injury.

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Organochlorine residues, eggshell thinning and hatching success in British sparrowhawks

SPARROWHAWKS (*Accipiter nisus*) in Britain have laid thin-shelled eggs throughout the period of DDT usage¹, and in several predatory species in North America an inverse correlation has been shown between shell thickness and DDE levels in eggs, both in natural and in experimental situations²⁻⁴. It has been claimed that eggshell thinning is associated with reduced hatching success, and that both effects could be implicated in a sequence leading to population decline. We have examined these relationships up to hatching stage in the sparrowhawk in Dumfriesshire, southern Scotland, where it still breeds in good numbers. During 1971–73 full details were obtained from 325 nests, and eggs from 130 clutches were collected for analysis.

Egg contents were homogenised; a 5 g aliquot was mixed with anhydrous sodium sulphate and sand, and extracted overnight in a soxhlet with hexane:acetone 2:1. The extract was evaporated to dryness in a tared beaker and total lipid estimated. The extract was then reconstituted in 25 ml hexane, and 5 ml was cleaned up by florisil column chromatography. (Liquid-liquid partitioning of the extract to remove fat was found to be unnecessary.) A further 5 ml aliquot was used for a duplicate estimate of lipid content. Hexane and hexane/ether chromatography fractions were used directly for analysis, using standard gas chromatography methods on columns packed with SE 30, SE 30-OV 210, and GE-XE 60 (after Richardson⁵). Results are given as parts per million (p.p.m.) in lipid, as the most valid basis for expressing organochlorine residues in eggs. On average, fresh sparrowhawk eggs contain approximately 6% by weight of lipid, so results can be converted to a wet weight basis by dividing them by 17.

The main residues found were DDE (the principal metabolite of DDT), polychlorinated biphenyls (PCB) and dieldrin (Table 1). Only results from eggs with embryos less than half grown were included. By this stage only a small reduction in lipid content (<10%) and negligible removal of calcium from the shell has occurred⁶. When eggs from the same clutch, but at different stages of development, were analysed, no evidence was found that residues were metabolised, as proposed by Hazeltine⁷. Residues remained in the same relative proportions, and the increase in concentration in lipid during development was of the extent predicted from the amount of lipid metabolised.

Table 1 Organochlorine content and shell indices of sparrowhawk eggs from southern Scotland 1971–73

	Mean ± s.e. (p.p.m. in lipid)	
DDE	148 ± 8	(n = 131)
PCB	66 ± 4	(n = 131)
Dieldrin	26 ± 1	(n = 131)
Total organochlorine	240 ± 11	(n = 131)
Shell index	1.17 ± 0.01	(n = 122)

Numbers refer to clutches. Each clutch represented once only by mean values and shell indices calculated by Ratcliffe's method¹.

Compared with 71 clutches collected in the same general area in 1900–45, which had a mean shell index of 1.43 ± 0.01 , those collected in 1971–73 showed an 18% reduction in shell index ($P < 0.001$). This is of the same order as that found for a wider part of Britain by Ratcliffe¹. Using all recent eggs, with each clutch represented only once by mean values, eggshell index was inversely correlated with the logarithm of DDE concentration (index = $1.568 - 0.186 (\log_{10} \text{DDE})$, $r = -0.359$, $P < 0.001$). It was also inversely correlated with $\log_{10}$ PCB concentration, though less well ($r = -0.231$, $P < 0.01$), and only poorly correlated with dieldrin concentration ($r = -0.101$, not significant). The correlation of PCB with shell thinning

in our eggs was probably because PCB was itself correlated with DDE ($r=0.480$, $P<0.001$). Lincer⁸, however, has shown that, while PCB had no direct effect on shell thinning in captive American kestrels, it potentiated the effect of DDE.

Among 325 study nests, 110 (34%) showed 'normal' hatching success, 101 (31%) showed partial success, and 114 (35%) failed completely. Success was classed as 'normal' if all eggs hatched, or if no more than one egg was addled (watery), and there were no egg breakages and no 'dead-in-shell' embryos. This criterion was established from extensive hatching data collected in Britain before 1946 (ref. 9). The hatching success of these various recent clutches was inversely related to their organochlorine content (Table 2).

Table 2 Hatching success and shell indices in relation to organochlorine content

Success	<i>n</i>	Total organochlorine content (mean $\pm$ s.e.)	<i>n</i>	Shell index
1. Normal*	17	157 $\pm$ 13	15	1.27 $\pm$ 0.03
2. Partial†	74	226 $\pm$ 11	70	1.20 $\pm$ 0.01
3. Nil	40	293 $\pm$ 25	37	1.11 $\pm$ 0.02

Each clutch represented once only by mean values; shell indices calculated by Ratcliffe's method¹.

* No more than one egg addled, no breakage and no 'dead-in-shell' embryos.

† One or more eggs broken or 'dead-in-shell' embryos but at least one young hatched.

The main cause of unsuccessful breeding was failure to lay (having built a nest). This occurred at 49 (15%) of 325 nests, and accounted for 43% of all complete failures. It was apparently not recorded in British sparrowhawks before 1946 (ref. 9). Egg breakage was the second commonest cause of failure, the 35 instances accounting for 11% of all clutches and 31% of all failures. Breakage was in general related to the degree of shell thinning (Table 3), but the facts that eggs in 22% of clutches with normal shell indices were broken, whereas eggs in 66% of those with low indices survived, imply that some other factor, such as parental care, was also involved. Usually the eggs in a clutch were broken individually over several days, until only one remained, which was then deserted. Analyses

breakage was a less important cause of unsuccessful breeding than failure to lay (having built a nest), while desertions and embryo deaths also accounted for a large proportion of failures in clutches. Clutches failing in all these ways, except desertions, showed organochlorine levels significantly higher than those showing normal success.

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Species diversity in temperate and tropical Ichneumonidae

ONE of the axioms of ecology is that the diversity of species is greater at low than at high latitudes. This was appreciated by Alfred Russell Wallace more than a century ago and has been repeatedly demonstrated in the more conspicuous groups of organisms such as trees, birds, and butterflies. Ecologists have produced a variety of theories, none entirely satisfactory, to explain latitudinal gradients in diversity, but an understanding of the phenomenon remains as elusive today as in Wallace's time.

Here we report an apparent exception to the rule in a family of parasitic Hymenoptera, the Ichneumonidae, which are mostly small, wasp-like insects. There are, however, more species of Ichneumonidae than of vertebrates, and in few groups of animals can there remain so many undescribed species. Virtually all ichneumonids are parasitic on the larvae and pupae of insects with a complete metamorphosis, especially Lepidoptera, Diptera, Neuroptera, Coleoptera, and Symphyta. The dependence of ichneumonids on herbivorous insects indicates that they have an enormous potential, as yet hardly explored, for programmes of biological control of pests.

Ichneumonids are abundant wherever there is vegetation and where it is not too dry, but few species are common. We have obtained large samples of them from three gardens: at Kampala in Uganda, Freetown in Sierra Leone, and Leicester in England. We have available another sample collected in Skåne, southern Sweden, not from a garden, but from disturbed land along a small stream. All four samples were collected in Malaise traps¹, tent-like constructions into which flying insects wander to fall eventually into a bottle of alcohol attached to the top of the trap. Malaise traps employ neither bait nor light and do not therefore attract insects, although undoubtedly some insects are more likely to be caught than others because of their behaviour. The only assumption we have made in our calculations of species diversity is that the traps do not select for either common or rare species.

Table 3 Incidence of egg breakage* in clutches with different shell indices

Shell index	0.80-1.00	1.01-1.20	1.21-1.40	1.41-1.60
No. of clutches	9	52	57	3
No. with breakage	7	20	12	1

* One or more eggs broken; clutches deserted fresh excluded.

of those remaining eggs from clutches gave organochlorine levels of 350 ± 47 p.p.m. The third commonest cause of failure was desertion, which occurred at 5% of all nests and accounted for 15% of all failures. The 16 clutches involved showed a wide range of organochlorine contents, with a mean of 200 ± 23 p.p.m., lower than that in other types of complete failure. The remaining 13 clutches were unproductive because the eggs, though incubated and undamaged, failed to hatch. This involved 4% of all nests, and 11% of all failures. Such failures might have been due to poor parental care, impaired gas and water exchange through a thin shell or a direct toxic effect of high residue levels. Embryo deaths from thin shells alone seems unlikely, as the 10 clutches involved had by no means the thinnest shells (mean 1.15 ± 0.01), but by far the highest organochlorine levels (mean 363 ± 19 p.p.m.). Among nests which produced at least one young, unhatched eggs were found in 51 (19% of all nests), broken eggs in 27 (8% of all nests), and both unhatched and broken eggs in another 23 (7% of all nests). Once the eggs hatched, survival of young in all groups of nests was greater than 90%.

In conclusion, in spite of widespread shell thinning, egg

The three gardens are not of course ecologically identical—Kampala and Freetown are on opposite sides of tropical Africa; the garden at Leicester is in the suburbs of a large city—nevertheless they share features common to all gardens: an abundance of flowers, and a wide variety of plants and trees introduced from other parts of the world. The Skåne sample should perhaps be considered separately and is added only to provide further evidence of the pattern of species diversity discovered.

Results of an analysis of the four samples are given in Table 1. The traps were operated day and night in all weather, and the sampling period in each locality included all months in which insects are flying. The differences in sample size shown in Table 1 do not necessarily reflect differences in abundance; much depends on the habitat and the efficacy of the traps in different situations, but we have the impression that, as a group, ichneumonids are rather more abundant in the temperate than in the tropical localities. The number of species recorded in each of the four samples is astonishing; the figure of 326 species in the Leicester garden represents about 16% of the species on the British list².

The information theory index of diversity³, H , enables an overall comparison of the four samples. The values of H for Freetown and Leicester are not significantly different but all other paired samples are different from each other at the 1% level. The value of H is significantly smaller for the Kampala than for the Leicester and Skåne samples and is smaller for the Freetown than for the Skåne sample.

The massive species diversity in the Ichneumonidae is further reflected in Table 1 by the calculation of the number of species taken once only and the percentage of the commonest species in each of the four samples. A quite extraordinary number of species was found once only and no species is really common.

Table 1 Two tropical and two temperate samples of Ichneumonidae compared

	Sample size	Species	Diversity index, H , and s.e.	Species taken once	% commonest species
Kampala	2,268	293	4.524 ± 0.032	116	10.1
Freetown	1,979	319	4.934 ± 0.029	117	4.9
Leicester	2,495	326	4.937 ± 0.024	122	3.2
Skåne	10,994	758	5.481 ± 0.014	203	5.5

There is no evidence of the expected greater diversity in the two tropical localities. A large (but for the tropical species, unknown) proportion of the species of ichneumonid parasitise Lepidoptera caterpillars. There is no doubt about the greater diversity of Lepidoptera in the tropics. We estimate that there are 300 species of butterflies in the Freetown garden⁴ but have found only 15 species in the Leicester garden. Butterflies comprise only a small fraction of the total species of Lepidoptera but probably reflect the situation in the remainder. The explanation of the high diversities in both the tropical and the temperate samples is probably associated with the fact that each species of ichneumonid tends to be niche specific rather than host specific⁵. In the parasite-host relationships considered here there may be no more niches in the tropics than in the temperate region, in contrast to the situation that probably prevails for most other feeding relationships.

We suggest on the basis of these samples that the Ichneumonidae are an exception to the rule that species diversity decreases from the equator to the poles, and that this single, little known family of insects can produce, even within a small area, a variety of species unparalleled in any other family of organisms. The Leicester sample is from an ordinary suburban garden. The trap sampled insects flying below 1.1 m over an area of only 2.6 m² and yet in one season produced 326 species, seven times as many as have

been recorded in Monks Wood National Nature Reserve⁶. Both tropical samples contain many species and some genera new to science⁷ and the entire collection has been given to the American Entomological Institute.

We thank Drs Henry and Marjorie Townes of the American Entomological Institute for tabulating the species. We also thank D. O. Chanter for computing the diversity values and Bo Svensson for operating the Malaise traps in Sweden.

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Role of antennae of the dragonfly *Orthetrum cancellatum* in flight control

THE function of the antennae of insects as air current sense organs and their relationship to flight control has already been investigated by behavioural studies in flies, bees and locusts¹⁻⁶, all of which are excellent fliers. The dragonfly is also an excellent flier although it possesses a very different flight apparatus⁷⁻⁹. Even though flight is apparently influenced by the eyes and the neck sense organs of the dragonfly^{10,11}, it seems unlikely that these organs have anything to do with the control of movements which are dependent on the surrounding air for their stimulation. For the measurement of flying speed relative to the surrounding air, for example, one might expect that, just as in the other insects, the antennae play an important and specific role. It is this hypothesis which has been tested in the present work, the results of which are reported below.

Experiments were carried out on the dragonfly *Orthetrum cancellatum* L. (Anisoptera, Odonata) 2-6 d after the imaginal moulting of larvae, which had been collected from various ponds in the area. For the flight experiments the foremost dorsal part of the mesepisterna of the animal was firmly fixed to a rod by the application of rosin wax. The wing-stroke angles, ϕ (Fig. 2a) of the fore and hindwings of the animal flying tethered in front of a wind tunnel were measured from photos (red light; exposure time, 1 s) which were taken just after the start of flight. The optical axis of the camera was perpendicular to the wing-stroke plane which formed an angle of about 45° with the vertical. The room temperature was 29° C. Control measurements at the end of each experiment showed that the animals were not noticeably exhausted.

The two antennae of the dragonfly are relatively small and are located in the antennal sockets between the frons, the vertex and the compound eyes (Fig. 1). Each antenna is composed of the basal small conical scapus, the somewhat elongated cylindrical pedicellus and the distal bristle-like flagellum pointing frontally, upwards and laterally. It is the scapus-pedicellus joint which is primarily responsible for active movements of the antenna even though passive

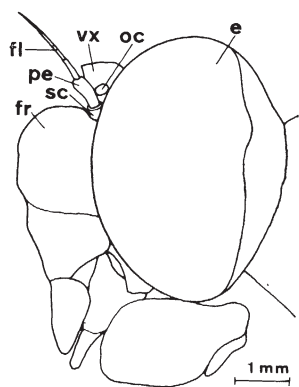


Fig. 1 Lateral view of the head of *Orthetrum cancellatum* (♀). The antenna consists of the scapus (sc), pedicellus (pe) and flagellum (fl). e, Eye; fr, frons; oc, ocellus; vx, vertex.

movements can be effected by external forces in the joints between the four flagellar segments as well as in the pedicellus–flagellum joint. For example, during flight, the flagellum is bent backwards at these joints by the impact pressure from the frontal air current. It is possible that it is this movement which stimulates mechanoreceptors situated at these joints (for example, Johnston's organ located in the pedicellus) and which consequently may allow the dragonfly to register its flight speed relative to the surrounding air, as already shown in other insects^{1–6}. To test this hypothesis, the flight behaviour of normal insects and insects without flagellae was compared, using the total wing-stroke angles (φ_t) of the fore and hindwings as behavioural flight parameters. The dorsal (φ_d) and ventral components (φ_v) of the wing-stroke angles were also evaluated as shown in the insert of Fig. 2a $\varphi_t = \varphi_d + \varphi_v$.

In normal animals, the total wing-stroke angles (φ_t) of the fore and hindwings in the absence of air currents are approximately of the same value (Fig. 2a and d). These wing-stroke angles (φ_t) are reduced equally in both pairs of wings as air speed increases (Fig. 2a: n ; $\Delta\varphi_{t0-4} = 48^\circ$. Fig. 2d: n ; $\Delta\varphi_{t0-4} = 40^\circ$, $P = 0.18$). This decrease depends only on the reduction of the dorsal component (φ_d ; Fig. 2b, e: n) since the ventral component (φ_v) is nearly independent

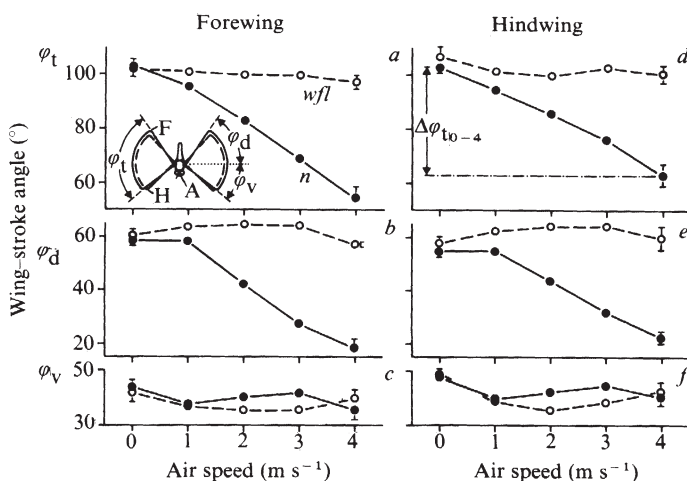


Fig. 2 a–f, Dependence of the wing-stroke angle (φ) on the air speed of a wind tunnel. Each point represents the mean of 70–110 measurements from 11 females and 8 males. Vertical bars indicate the standard error ($\pm$ s.e.). A, antenna; F, forewing; H, Hindwing; n , normal animals; wfl , animals without flagellae; φ_t , total wing-stroke angle; φ_d (φ_v), dorsal (ventral) component of the wing-stroke angle; $\Delta\varphi_{t0-4}$, difference between the wing-stroke angle without air current (0) and with frontal air current with a speed of 4 m s⁻¹ (ref. 4).

of the air speed (forewing: Fig. 2c: n , $\Delta\varphi_{v0-4} = 8^\circ$; $P = 0.08$. Hindwing: Fig. 2f: n ; $\Delta\varphi_{v0-4} = 7^\circ$; $P = 0.045$).

On the contrary, in insects whose flagellae had been cut off at their base 30 min or more before the experiments, the decrease of the wing-stroke angles of the forewings with increasing air speed ($\Delta\varphi_{t0-4} = 7^\circ$; Fig. 2a: wfl) is much smaller than in normal insects ($\Delta\varphi_{t0-4} = 48^\circ$; Fig. 2a: n ; $P < 0.0002$). This is also true for the wing-stroke angles of the hindwings (Fig. 2d: wfl , $\Delta\varphi_{t0-4} = 6^\circ$; n , $\Delta\varphi_{t0-4} = 40^\circ$, $P < 0.0002$).

It seems, then, that the different flight behaviour of normal insects and those without flagellae is reflected by the distinct change in the dorsal component of the wing-stroke angle (φ_d). More specifically, the decrease in dorsal component (φ_d) as a function of increasing air speed is much reduced in both the forewing (Fig. 2b; $P < 0.0002$) and the hindwing (Fig. 2e; $P < 0.0002$) of dragonflies without flagellae. The ventral component (φ_v) of both wing pairs, however, is independent of the air speed both in animals without flagellae and normal animals (Fig. 2c, f).

The results indicate that some mechanoreceptors in the antennae, stimulated during flight by the action of air currents on the flagellae, are responsible for relaying information about the air speed necessary for the control of wing movements. These receptors, however, affect only the dorsal wing-stroke angle, the ventral components of both wings functioning independently of the surrounding air. That means that the antennae of dragonflies may be air-current sense organs which control flight performance, probably the flying speed, a finding which is consistent with those previously reported for other insects^{1–6}.

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Evidence for a gene-specific RNA determining resistance in wheat to stem rust

ALTHOUGH the inheritance of resistance¹ in wheat (*Triticum aestivum* L.) to stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.) is fairly well understood, most efforts to elucidate the biochemical mechanism involved have been unsuccessful. Biochemical studies of plant diseases involving rusts and other obligate parasites² have contributed to knowledge of biochemical symptomatology, but no chemical changes have been correlated with the action of specific genes for resistance. Future advances it seemed,

would depend on a reliable test to facilitate such correlations, and so we have developed a bioassay with the necessary specificity, which detects the presence of metabolites involved in the expression of the resistance. We have thus been able to demonstrate a gene-specific RNA that seems to be directly responsible for initiating the resistant reaction in wheat against stem rust. A preliminary report of some of these findings has been presented³.

In our work with the wheat/rust system we were interested in the highly specific interactions that are consistent with the gene-for-gene hypothesis¹. Briefly, this hypothesis states that for every gene for resistance in the host there is a specific and related gene for virulence in the parasite. Interaction between these genes may involve transfer of information across the host/parasite interface. The substances involved in this transfer must be able to carry a high informational content because many genes for resistance have already been identified. Nucleic acids, proteins and complex carbohydrates fulfil this requirement. We believed that compounds involved in the expression of resistance could best be obtained from resistant reacting tissues containing a large number of haustoria, for histological observations² of the resistant interaction have shown that cell necrosis, which is characteristic of resistance, is always associated with the establishment of haustoria. To obtain such tissue we exploited the temperature lability⁶ of the *Sr6* gene at temperatures above 25° C. Wheat leaves containing this gene were inoculated with spores of the avirulent race C17 (56), and maintained at 26° C to suppress host cell necrosis that would normally occur in this interaction at lower temperatures. Seventy-two hours after inoculation, when the leaves were extensively colonised, plants were transferred to 20° C for a further 30 h to initiate the necrotic reaction, then collected for the preparation of extracts.

In developing a useful bioassay it was desirable to simulate natural conditions as closely as possible. Normally, in interactions involving the *Sr6* gene for resistance and the *P6* gene for avirulence, necrotic cells develop at most infection sites shortly after haustorial formation, whereas few necrotic cells are produced in susceptible interactions⁵. Our objective was to test extracts for their ability to induce the formation of similar necrotic cells in wheat leaves inoculated with a virulent race of stem rust. We defined an active extract as one that induces significantly more necrotic cells in the genotypically resistant leaves (R line) than in the genotypically susceptible leaves (S line) of wheat.

For statistical purposes, necrotic sites consisting of one or several contiguous necrotic cells were counted. If the average incidence of necrotic sites is the same in leaves of both lines, then we would expect, if we had equal numbers of observations in both cases, that half the necrotic sites would appear in the sample of the R line. This property can be used as the basis for a statistical test⁷ which has certain optimal properties if the necrotic site counts follow a Poisson law. If N_R and N_S necrotic sites occur in n_R and n_S observations in leaves of the R and S lines, respectively, then we can use the properties of the binomial distribution to test if N_R necrotic sites occur in a total random sample of $(N_R + N_S)$ necrotic sites with probability $n_R/(n_R + n_S)$ expected if the average incidence of necrotic sites is the same in leaves of both R line and S line. For $N_R + N_S > 20$, a normal approximation was used.

Preliminary experiments with crude nucleic acid preparations showed that significantly more ($P < 0.005$) necrotic sites were produced when the extract obtained from the *Sr6/P6* interaction was injected into bioassay plants of the R line (containing the *Sr6* gene), than when it was injected into plants of the S line (Fig. 1). Non-injected leaves of either line, and leaves that were injected but not infected, contained virtually no necrotic sites. The presence of

necrotic sites in infected leaves of the S line suggested that the crude nucleic acid extract also contained substances which caused nonspecific necrosis in infected leaves of both lines, in addition to the component that showed specificity towards the *Sr6/p6* interaction.

Typically, crude nucleic acid extracts contained RNA, DNA and protein in a ratio of 5:4:1, by weight. Because of their high DNA content these crude extracts were viscous and difficult to inject. Treatment with cetyltrimethylammonium bromide (CTA), DNase and protease yielded preparations with higher specificity towards bioassay plants

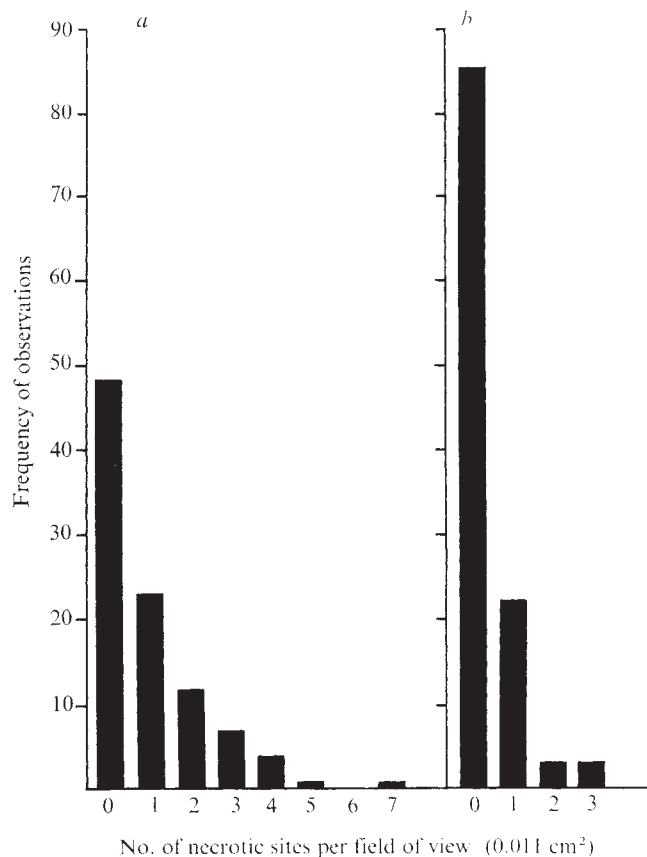


Fig. 1 Bioassay of a crude nucleic acid extract prepared from Chinese Spring wheat (containing the *Sr6* gene for resistance) infected with race C17(56) of wheat stem rust (containing the *P6* gene for avirulence). The extract was prepared⁸ by homogenising plant material with *n*-butanol/chloroform/1M K_2HPO_4 (1:1:2 v/v/v), followed by deproteinisation of the aqueous phase with phenol. Nucleic acids were recovered by precipitation with ethanol and redissolved in 15 mM NaCl, 1.5 mM sodium citrate, pH 7.0 (1/10 SSC). Primary leaves of bioassay plants (7-d-old) were heavily infected with a virulent race (C45(56A)) to give 800–1,600 appressoria cm^{-2} . Uredospore suspensions (6 mg ml^{-1}) in 0.1% gelatin and 0.001% Tween 20 were applied with cotton swabs along 2 cm of the adaxial (upper) leaf surface, 2–3 cm from the leaf tip. Inoculated plants were incubated in the dark (16 h, 20°C) then transferred to a growth chamber (16 h 20,000 lux; 8 h darkness; 20°C). This resulted in near-synchronisation of fungal development⁹. Twenty-four hours after the beginning of the first photoperiod, extracts in 1/10 SSC were injected⁹ into leaves between the tip and the inoculated area, so that the intercellular spaces in the inoculated region were flooded with extract. Six hours later the infected regions were excised, cleared and stained with Trypan blue (C.I. No. 23850) for microscopic examination⁵. Necrotic cells appeared to have collapsed and stained dark blue. They were also identified in unstained tissue by their characteristic fluorescence¹⁰. Resistant and susceptible near-isogenic lines of wheat produced by W. Q. Loegering¹¹ from chromosome substitution lines of Chinese Spring wheat were used in these studies. *a*, R line (*Sr6/p6* interaction); mean number of necrotic sites per field of view was 1.0 *b*, S line (*sr6/p6* interaction); mean number of necrotic sites per field of view was 0.246.

Table 1 Characterisation of the active component in a crude extract from resistant reacting wheat leaves (*Sr6/P6* interaction)

Treatment of crude extract	A_{260}	Chemical analysis ($\mu\text{g ml}^{-1}$)			Necrotic sites per cm^{-2} leaf of bioassay plants		
		RNA	DNA	Protein	R line (R)	S line (S)	R/S
None	40	1074	888	188	97.4	62.6	1.6**
Cetyltrimethyl-ammonium bromide (CTA) precipitation ¹²	40	1064	968	53	20.2	7.6	2.7**
CTA precipitation followed by DNase*	86	1780	220§	—	56.0	18.6	3.0**
	66	1369	169§	—	44.4	12.2	3.6**
CTA precipitation followed by DNase and protease†	70	—	—	384¶	48.5	8.1	6.0**
CTA precipitation followed by RNase‡	41	0	2440	—	8.5	20.2	0.4**

Details of the preparation of the crude extract are given in the legend to Fig. 1.

RNA and DNA were determined with ethidium bromide¹³ using highly polymerised calf thymus DNA (Sigma) and wheat germ RNA (Calbiochem) as standards. Protein was determined with Folin reagent¹⁴ using bovine serum albumin as standard. Plants used for the bioassay were: for R line, Chinese Spring wheat containing the *Sr6* gene; and for S line, Chinese Spring wheat containing the *sr6* gene. Both were infected with race C45(56A) of wheat stem rust (containing the *p6* gene).

* We used 100 μg of RNase-free DNase (Worthington) and 30 A_{260} units nucleic acid ml^{-1} , 500 mM NaCl, 50 mM MgCl_2 , 10 mM Tris-HCl (pH 7.4) 1 h, 20° C. After incubation the mixture was deproteinised with phenol, extracted five times with ether, and then nucleic acid precipitated with ethanol and dissolved in 1/10 SSC.

† We used 10 mg of predigested insolubilised protease (Miles) and 30 A_{260} units nucleic acid ml^{-1} , in 1/10 SSC, 1 h, 37° C. Protease was removed by centrifugation, nucleic acid precipitated with ethanol and dissolved in 1/10 SSC.

‡ We used 100 μg pancreatic RNase (Sigma), 25 units T_1 RNase (Miles) and 30 A_{260} units nucleic acid ml^{-1} , 1 h, 37° C. Enzyme was removed with phenol as described above.

§ Oligonucleotides, as determined by acrylamide gel electrophoresis.

¶ Apparent high protein content due to products from self-digestion of protease.

** R significantly greater than S at 0.05 level of probability.

of the R line (Table 1). Digestion with RNase, however, destroyed this specificity, indicating that the active component was a ribonucleic acid. CTA precipitation of the crude nucleic acid, followed by digestion with DNase, resulted in preparations containing RNA, oligonucleotides of DNA and protein in a ratio of 94:5:1, by weight. Such preparations had a low viscosity at high RNA concentrations, and could be readily injected into wheat leaves. Eight

RNA is directly involved in the resistant reaction of wheat to stem rust and that the active RNA shows specificity comparable to the specificity of the host/parasite system. Our bioassay should make it possible to obtain further information on the chemical nature of the active RNA and its production, and on the mechanism of action leading to cell necrosis. We believe that this approach to the study of host/parasite interactions may be applicable to other host/

Table 2 Specificity of RNA from rust-infected resistant reacting wheat leaves towards host genes for resistance

Host-parasite complex from which RNA extract was obtained		Bioassay system			Necrotic sites per cm^{-2} leaf of bioassay plants		
Gene interaction	Avirulent race* used	R line	S line	Virulent race* used	R line (R)	S line (S)	R/S
<i>Sr6/P6</i>	C17(56)	<i>Sr6/p6</i>	<i>sr6/p6</i>	C45(56A)	15.9	9.1	1.8†
		<i>Sr5/p5</i>	<i>sr5/p5</i>	C45(56A)	3.9	5.8	0.7
<i>Sr5/P5</i>	C5(29-1)	<i>Sr5/p5</i>	<i>sr5/p5</i>	C11(15B-4)	3.6	3.6	1.0
		<i>Sr6/p6</i>	<i>sr6/p6</i>	C45(56A)	1.0	5.6	0.2
<i>Sr6/P6</i>	C18(15B-1L)	<i>Sr6/p6</i>	<i>sr6/p6</i>	C22(32)	9.5	3.2	3.0†
		<i>Sr11/p11</i>	<i>sr11/p11</i>	C22(32)	1.8	3.0	0.6
<i>Sr11/P11</i>	C21(32)	<i>Sr11/p11</i>	<i>sr11/p11</i>	C22(32)	4.9	2.1	2.3†
		<i>Sr6/p6</i>	<i>sr6/p6</i>	C22(32)	2.9	3.7	0.8

RNA was purified from crude extracts using CTA precipitation and digestion with DNase (see Table 1).

* C numbers refer to Canadian designations of stem rust races¹⁵; numbers in brackets refer to standard race designations¹⁶.

† R significantly greater than S at 0.05 level of probability.

separate RNA extracts prepared in this way were tested in this bioassay system and they all caused more necrosis in plants of the R line than of the S line.

Having demonstrated that an active RNA can be obtained from the *Sr6/P6* interaction, we wished to know if active extracts could be obtained from other gene interactions, and whether active RNA isolated from one system was active in systems containing other genes. An active RNA extract obtained from the *Sr6/P6* interaction was not active in the *Sr5* bioassay system (Table 2). When a separate extract prepared from leaves of the *Sr6* R line, infected with a different race (avirulent to *Sr6*) was used, it was active in the *Sr6* bioassay system but inactive in the *Sr11* bioassay system. Conversely, an extract from leaves of the *Sr11* R line, maintained at 20° C and infected with a race avirulent to *Sr11* (*Sr11/P11* interaction), was active in the *Sr11* bioassay system but inactive in the *Sr6* bioassay system. It was not possible to obtain an active extract from the *Sr5/P5* interaction. We attributed this lack of activity to the fact that the host/parasite interaction conditioned by the *Sr5* gene is usually restricted to very few cells and permits little potential for the production of detectable amounts of an active component. Since the *Sr5* gene is not temperature sensitive, it is not possible to obtain more extensive colonisation of the leaves by growing the inoculated plants at higher temperatures.

In summary, our experiments have demonstrated that

parasite systems for which a gene-for-gene relationship exists.

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Virus-like particles in *Leishmania* parasites

VIRUSES or virus-like particles (VLPs) have been found in *Entamoeba histolytica*¹, in the amoeboflagellate *Naegleria gruberi*² and in malaria parasites³⁻⁵, but there have been no reports as yet of such organisms being found in the Trypanosomatidae. During investigations on the ultrastructure of various members of the genus *Leishmania*, however, VLPs have been found in the promastigote forms of an isolate of *Leishmania hertigi*. *Leishmania hertigi*⁶ was obtained from Panama and kept in our laboratory on blood agar slopes or as cryopreserved material at the temperature of liquid nitrogen. Examination of the promastigotes of *L. hertigi* immediately revealed cytoplasmic inclusions of a type which had not been observed in any of our previous studies on the promastigotes of *Leishmania* species in culture.



Fig. 1 Electron micrograph of section of promastigote of *L. hertigi* showing group of VLPs (V), and hollow core of some of the particles can be seen. Material from 6 d culture fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide and embedded in Araldite. K, Kinetoplast; m, mitochondrion; N, nucleus ($\times 22,000$).

The particles are distributed throughout the cytoplasm of the promastigotes (Figs 1 and 2), and are seen in a high proportion of sections examined under the electron microscope. It is likely therefore that all parasites of this isolate are infected. The particles have no effect on the growth pattern or division of the *Leishmania* promastigotes in culture and no apparent detrimental effect on cell structure. The promastigotes divide by longitudinal binary fission in the cultures and the particles are present in the two halves of the dividing parasite and are maintained in this way. The individual particles are usually spherical in section 55-60 nm in diameter and packed in a manner typical of virus organisation, (Figs 1, 2, and 3). There is an electron-dense outer ring and an electron-lucid core, 17-20 nm in diameter (Fig. 3). The number of particles in each group is variable.

Within the cytoplasm of parasites which are known to contain VLPs, unusual rod-like structures are found which are not usually seen in trypanosomatid cells. The presence of VLPs may induce the production of these inclusions. It is not known if *L. hertigi* acquired the VLPs in natural conditions from a sandfly vector or from the mammalian

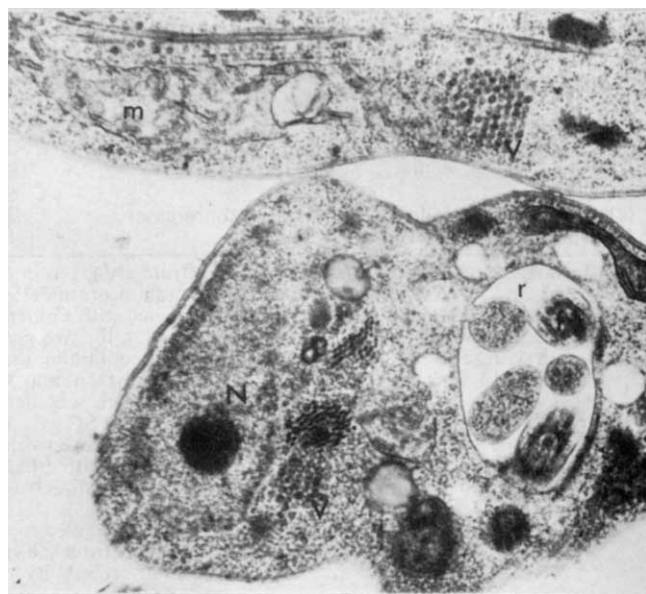


Fig. 2 Section through two promastigotes of *L. hertigi*; both parasites show VLPs (V) in their cytoplasm. Unusual cytoplasmic inclusions are present in both these parasites and may be related to the presence of VLPs. Fixation as in Fig. 1. r, Reservoir; f, flagellum ($\times 22,000$).

host, the tropical porcupine *Coendou rothschildi*. It is unlikely the VLPs were acquired in laboratory conditions as no other *Leishmania* isolates examined have been found infected, but even if the parasites were infected in the laboratory such an observation in itself would be of great interest.

Further studies on these inclusions are in progress to characterise their nature and to determine if they are infective to other species of *Leishmania* and *Trypanosoma* and to mammalian cell cultures. The effects of any virus on the parasite genome have obvious evolutionary implications. It is also possible that intercurrent virus infections may play a hitherto unrecognised role in the clinical manifestations of *Leishmania* and *Trypanosoma* infections. Relatively few isolates have been examined with the electron microscope and such associations may be common with resulting important clinical and chemotherapeutic implications, both in treatment and possibly in transfer of drug resistance between parasites.

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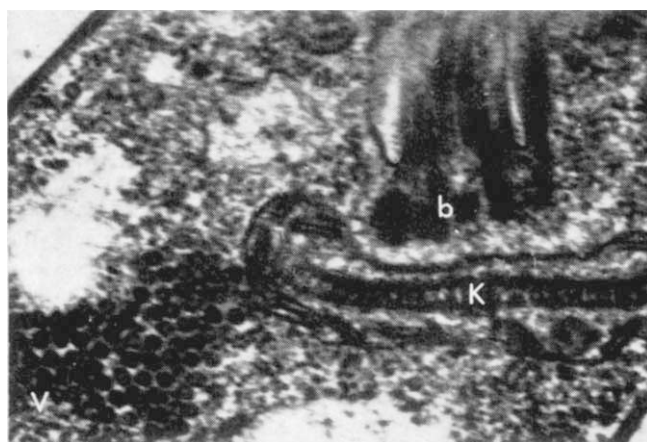


Fig. 3 Showing aggregation of VLPs adjacent to the kinetoplast (K) of *L. hertigi*. The dense outer layer and electron lucid core can be seen in some particles. Fixation as in Fig. 1. b, Basal body ($\times 120,000$).

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Devastation of a fringing coral reef by *Acanthaster*

A RECENT synopsis of reports on the status of populations of the Crown of Thorns Starfish, *Acanthaster planci* (L.), in the Indo-West Pacific¹, indicates that no extensive destruction of living corals by this starfish has yet been recorded in the western Indian Ocean. Here I present a preliminary account of an aggregated starfish population and extensive mortality of hard corals, observed in May 1973 off the north coast of Grande Comore, Comoro Islands.

Examination of a portion (about 150×300 m) of the fringing reef off Lac Salé, north-east Grande Comore (at 11° 21'S, 43° 22'E), showed an approximate 90% mortality of hard corals; this was estimated by eye at four points on the reef slope as the percentage of recently dead coral (skeletons both white and various shades of green, being in different stages of colonisation by green algae) over total coral (recently dead plus still living coral).

That the mortality was due to *Acanthaster* was inferred from the presence on the Lac Salé reef of large numbers of these starfish and from the discovery of many more at Mitsamiouli on the north-west corner of Grande Comore, where an estimated 40% of the hard corals had recently been killed. Coral predation became less frequent as I moved to the south off Mitsamiouli; my visit to Grande Comore coincided, therefore, with the recent devastation of the Lac Salé reef and the incipient devastation of the Mitsamiouli reef. The indication was that a large number of *Acanthaster* had moved as a front in an anti-clockwise direction around the northern coast of Grande Comore. Thus, the starfish that I found at Lac Salé would only have been the remnants of this mobile population. The survival of many corals in shallow water and of a small percentage of them on the reef slope, which was dominated by 'table' forms of the genus *Acropora*, rules out freshwater run off or upsurge of cold water as reasons for mortality, and further, nearly all the *Acropora* tables showed no sign of mechanical damage, although denuded of their tissue; thus changes in purely physical parameters cannot explain this mortality. A similar level of hard coral mortality due to *Acanthaster* has been observed on other badly affected coral reefs^{2,3}.

Hard coral colonies on the reef slope survived especially in the wider, outer parts of the surge channels which dissect the reef slope; here the coral colonies were separated from the main reef by a sandy floor, which may give partial protection from *Acanthaster*⁴. Many colonies also had been only partly denuded of their living tissue. Surviving colonies and surviving patches of partially denuded colonies are probably important in reef recovery⁵.

The number of fish species recorded by sight on this reef was consistent with values for other western Indian Ocean island reefs where little or no evidence of *Acanthaster* was found (Table 1). Out of a total of 20 species of chaetodontids, which are typical 'coral fishes'⁶, recorded by sight at the 3 localities, there were 13 at Lac Salé (90% hard coral mortality), 12 at Iconi (hard corals uncommon), and 12 at Mitsamiouli (40% hard coral mortality in places). Thus while *Acanthaster* and other factors can have a destructive effect on the hard coral cover, they may not affect the number of fish species. Porter⁷ found that *Acanthaster* predation can have a beneficial effect on coral species diversity. Heavy stressing of Grande Comore reefs by bottom fishing and spear fishing inhibits more detailed analysis of fish faunal differences between the affected and unaffected reefs; certain species, however, such as the herbivorous acanthurids⁸ *Ctenochaetus striatus* and *Acanthurus leucosternon*, may have increased on the Lac Salé reef.

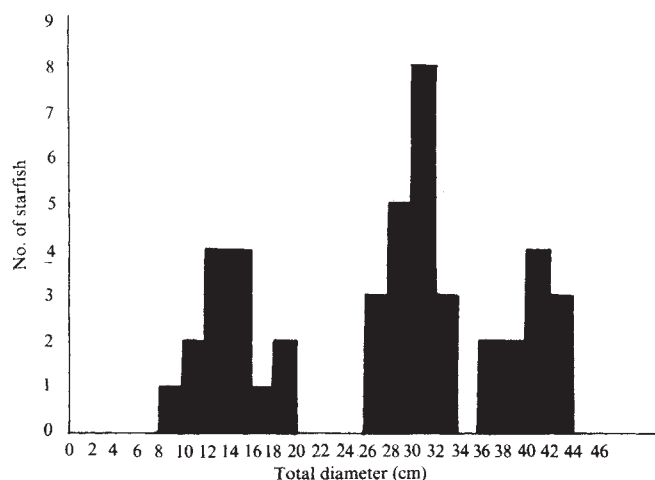


Fig. 1 Size-frequency histogram (total diameter) of 44 *Acanthaster planci* collected between 1300 and 1500 h on May 24, 1973 in the same reef flat habitat of the Lac Salé, Grande Comore, fringing reef.

Few *Acanthaster* were found in the zone of high hard coral mortality. Nearly all the individuals found were confined to the reef flat, where the coral cover was poor, but a higher proportion of the corals still survived. Conditions of swell were more marked on the reef flat than on the reef slope; starfish detached from the substrate on this reef flat had great difficulty in regaining their hold. No starfish were found in the act of feeding during the daytime, and very few individuals were visible from the sea surface. Small individuals were found only by breaking open clumps of branching corals. The size-frequency histogram (total diameter) for 44 starfish collected in the same reef flat habitat indicates the presence in the population of at least two size-classes (Fig. 1). The population may still be a mixed one, however, with animals contributed from the once-rich reef slope and from the impoverished reef flat; Dana *et al.*⁹ suggested that the bimodal size distribution reported for some Sudanese Red Sea *Acanthaster*¹⁰ might not be due to the presence of two age classes, and could have resulted from the sampling of different habitats, where coral food was more or less abundant.

H. R. Lubbock (personal communication, 1971) found large numbers of *Acanthaster* in September 1971 in Moroni harbour, Grande Comore, and from discussions with a local diver (J. Nicholas, personal communication) it seems probable that this starfish has been common on Grande Comore for even longer.

In addition to the 18 localities recorded in Table 1, I have examined nine others in the granitic Seychelles; only off Lac

Table 1 Eighteen western Indian Ocean island localities visited between April 19 and July 10, 1973

Locality	Observation time (h)	Maximum depth (m)	Fish species seen (No.)	<i>Acanthaster</i> : type and state of reef
Lac Salé, Grande Comore	3	9	104	Many starfish present; ~ 90% dead coral on reef slope. Fringing. Reef once rich.
Mitsamiouli, Grande Comore	1	13	73	Many starfish present; ~ 60% corals still living. Fringing. Reef still rich.
Iconi, Grande Comore	1	26	82	Three seen; almost no live hard corals. Fringing. Reef long 'dead'.*
Récif Vailheu, Grande Comore	1	37	85	None seen; no evidence of predation. Rich table reef.
Canzoni I., Moheli	1	26	87	None seen; no evidence of predation. Fringing. Rich coral in shallows.
Ouénéfou I., Moheli	1	19	106	None seen; no evidence of predation. Fringing. Rich coral in shallows.
Andrema I., Mayotte	4	34	154	One individual seen; no evidence of predation. Fringing. Very rich coral in shallows.
Banc du Geyser	1	28	80	None seen; no evidence of predation. Atoll. Rich coral on outer reef flat.
Banc Suzy, Nossi Bé	1	16	57	None seen; no evidence of predation. Patches.
Tsara Bajina, Mitsio Is.	2	6	97	None seen; no evidence of predation. Patches.
Behangovo, Mitsio Is.	0.5	28	43	Four seen; no evidence of predation. Rare patches on desolate slope.
Ankarea, Mitsio Is.	3	9	113	None seen; no evidence of predation. Patches.
Radam Is.	1	9	76	None seen; no evidence of predation. Patches.
Poivre, Amirante Is.	2.5	19	93	None seen; no evidence of predation. Atoll. Rich coral in places.
Alphonse, Admirante Is.	4	43	152	None seen; no evidence of predation. Atoll. Rich coral in shallows.
West North I., Cosmoledo	4	37	152	None seen; no evidence of predation. Atoll (elevated). Rich coral on outer reef flat.
Assumption	4	56	160	None seen; no evidence of predation. Elevated reef. Rich coral on outer reef flat in places.
Johnny Channel, Aldabra	1.5	9	84	None seen; no evidence of predation. Atoll (elevated). Some rich coral on channel edges.

Notes were taken underwater.

*No evidence for implication of *Acanthaster*.

Observation time is given to the nearest half hour. Also recorded are maximum depth reached, number of fish species recorded by sight and sightings of *Acanthaster*, with notes on the type and state of the reef.

Salé and Mitsamiouli, Grande Comore, have I found large numbers of *Acanthaster* and an extensive, recent mortality of hard corals. There is evidence, however, of recent population increases of *Acanthaster* elsewhere in the western Indian Ocean: on a reef off Mauritius¹, on Kenya reefs (ref. 1 and R. Tauber, personal communication) and on the 'Grand Récif' off Tuléar, Madagascar (L. A. Mauge, personal communication). It is interesting to note that de Lorient¹¹ reported in 1885 that specimens of *Acanthaster* were *assez fréquents* (quite common) in Mauritius.

There is no doubt that aggregations of *Acanthaster* can be responsible for extensive mortality of reef corals. Recent studies have shown that high levels of reef coral predation may be concomitant with active reef growth¹², and that in the case of patch reefs in Bermuda¹³, a phase in their development is burial by sufficient particulate sediment to kill the sessile reef organisms. Aggregations of *Acanthaster* are destructive in the short term, but they may be an important factor in coral reef development in the long term^{5,9,12,14,15}.

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Rats learning to work for alcohol

IF animals are to be used in the study of alcoholism, it is important that they show characteristics similar to human alcoholics. It has been demonstrated that animals can show tolerance and withdrawal symptoms to alcohol¹: therefore, animals can legitimately be used to study these aspects. There has, however, been little evidence that animals develop the most important factor in alcoholism, a strong motivation to obtain alcohol for drinking. As there have been no demonstrations of animals learning a new response, or even continuing to make a previously learned response, to obtain drinking alcohol, in the presence of food and water *ad libitum*, it has been possible to claim that their alcohol consumption is 'accidental and inadvertent'². If this were true, experiments on voluntary alcohol drinking by animals would be of little value for generalising to humans.

It therefore seemed critical to determine if animals could learn to work for drinking alcohol and to assess their motivation, if any, for alcohol. Animals which had developed a strong preference for alcohol in a free-choice situation were thought to be most likely to be motivated to obtain alcohol. Therefore, 60 male albino rats of three different strains were given at least 55 d free access to a 10% (v/v) ethanol solution, tap water and the standard Alko rat food. From these, six were selected for testing in the Skinner box because of their consistent high preferences for alcohol. Three were of the AA strain developed for high alcohol consumption^{3,4}, two were Sprague-Dawleys, and one was of the ANA strain^{3,4}. During the week before testing, their ratios of ethanol solution to total fluid consumption ranged from 0.60 to 0.98.

These rats were then each housed continually in a standard two-bar Lafayette operant conditioning cage, modified

so that pressing one bar yielded a drop of about 0.1 ml of 10% alcohol solution, and pressing the other bar produced a similar drop of water. An open jar of food and a bottle of water were also available *ad libitum* at all times in the Skinner box. A 10 h dark, 14 h light illumination schedule was used.

On the first day of testing, before each rat was placed in the Skinner box, drops of water were placed on the water bar, and alcohol solution on the alcohol bar. No other shaping of any kind was used. A rat was arbitrarily considered to have learned to work for alcohol if it responded more than 100 times d^{-1} for alcohol and at the same time pressed at least twice as often for alcohol as for water.

All six rats met this criterion, four during the first night, one on the second night and one on the third night in the Skinner box. Once a rat had learned to work for alcohol, it continued to meet the criterion every day thereafter (Fig. 1.)

Subsequently, a reversal test was made by interchanging the positions of the alcohol and water bars. All but one of the animals learned the reversal: two during the first night, two on the second night, and one on the fourth night.

Two ANA rats which had not developed alcohol preferences^{3,4} were also selected from the original 60 animals for testing as controls in the Skinner box, using the same procedure as before. Neither rat met the criterion during 10 d of testing. One ignored both bars and obtained its fluid from the free water bottle. The other pressed both bars about 50 times per night, but still got most of its liquid from the free water bottle.

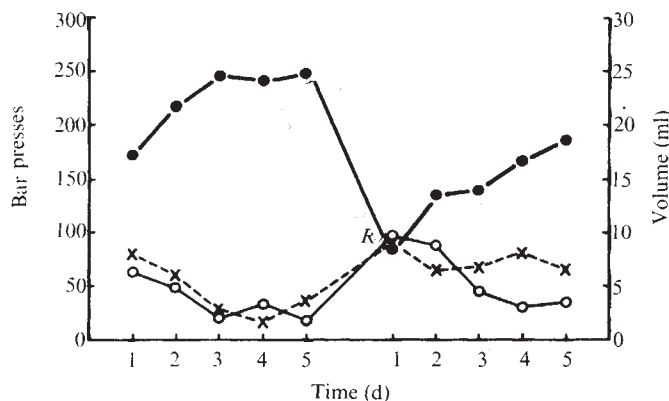


Fig. 1 Mean daily responding for 10% (v/v) ethanol solution (●) and for earned water (○) by six rats. As each bar press produced about 0.1 ml, the approximate volumes earned can be read from the ordinate on the right. Consumption of 'free water' (X) from a bottle available *ad libitum*, is also shown. After the 'reversal' (R) the alcohol and water bars were interchanged. The lower alcohol responding and higher free water intake at this time is caused by one rat that failed to learn the reversal.

The motivation for alcohol was tested in two of the rats which had previously learned to work for alcohol: one was an AA, weighing 421 g, the other a Sprague-Dawley, weighing 440 g. Weights were added progressively each day to the back of the alcohol bar, thus increasing the effort required to press it. On a continual reinforcement schedule, such as used here, this procedure should successfully measure motivation, since it has been found that rats otherwise prefer a bar requiring less effort to press⁵. This method was chosen rather than, for instance, electrifying the alcohol bar, because it should be less likely to produce confounding stressful effects.

Both rats continued pressing the alcohol bar until over 140 g were attached to it. The AA rat responded at a relatively constant rate until 137 g were attached, but showed a sharp decrease when additional effort was required. Responding by the Sprague-Dawley decreased continually through-

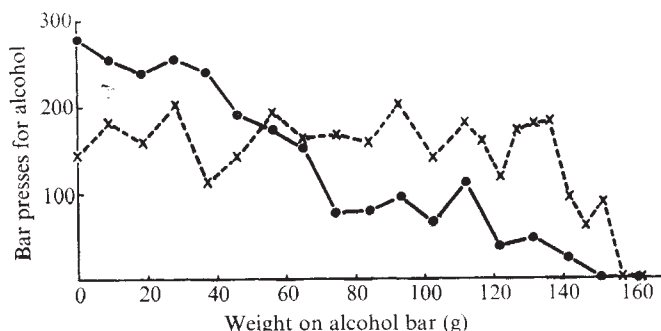


Fig. 2 Daily responses for 10% alcohol solution by rats SD15 (●) and AA141 (X), as a function of the effort required to press the alcohol bar. Each day progressively larger amounts of weight were attached to the alcohol bar, at a distance from the fulcrum equal to that of the portion upon which the rats pressed. Free food and water, and a bar, without weights, producing water, were continually available.

out the experiment as a nearly linear function of the weight attached to the alcohol bar (Fig. 2).

Unlike previous studies, in which rats have bar pressed for alcohol⁶⁻¹⁰, the present findings clearly cannot be attributed to motivation from hunger or thirst, as food and water were concomitantly available *ad libitum*. The inclusion of a bar producing water controls for spontaneous bar pressing or responding motivated by stimulus change or mastery. The reversal task controls for position preference. Since no shaping was used, the results are not a function of the skill of the experimenter in shaping procedures. Thus it seems that the learning was reinforced by obtaining alcohol *per se* to drink.

Some animals have previously been found to work for intragastric or intravenous injections of alcohol, and in a few cases to learn new responses for the alcohol injections¹¹⁻¹⁴. These results showed that alcohol could be a reinforcer, but they could not eliminate the possibility that the animal's motivation for alcohol injections was somehow different from the alcoholic's motivation for a drink of alcohol. As the present study shows that animals will also learn to work for drinking alcohol, it is less likely that a fundamental difference exists between the motivations of humans and these animals for alcohol.

From a comparison with two unsuccessful (and unpublished) previous attempts to train rats to work for alcohol, it seems likely that the present procedure was successful because, first, rats were used that had demonstrated long-standing, consistent, strong preference for the alcohol solution; second, the animals remained in the Skinner box during the night, which is when rats normally drink alcohol¹⁵, rather than being tested only in daytime sessions; and third, they had not previously been taught to bar press for water, a procedure which in one of the unsuccessful previous attempts seemed to produce a subsequent decrease in voluntary alcohol drinking.

In conclusion, humans are not the only creature sometimes motivated to obtain alcohol for drinking: some rats seem to develop a similar motivation which can lead them to learn new responses for alcohol reinforcement and to expend rather large amounts of effort to obtain it. These findings also suggest the possibility of opening many questions concerning alcohol drinking by animals to sophisticated analysis with the use of operant techniques.

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- ¹² Winger, G. D., and Woods, J. H., in *Alcoholism and the Central Nervous System* (edit. by Seixas, F. A., and Eggleston, S.), *Ann. N.Y. Acad. Sci.*, **215**, 162 (1973).
- ¹³ Woods, J. H., Ikomi, F., and Winger, G., in *Biological Aspects of Alcoholism* (edit. by Roach, M. R., McIsaac, W. M., and Creaven, P. J.) (University of Texas Press, Austin, 1971).
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GENERAL

Time as heard in speech and music

THE time intervals of consequence in music range from about 50 ms, the fastest that notes may be separately fingered on the piano, to about 1 s, the tempo of slow largo. Poetry is customarily recited with beats every 600–800 ms, and the semi-isochronous stresses of speech rhythm are, according to Classe, of the order of 600 ms apart¹. We take pride in the accuracy with which we can produce and appreciate these intervals or deviate from them at will. That we require an auditory information storage of brief duration, briefer than that customarily named short-term memory, is known, Neisser calling it echoic memory²; but, as distinct from our other kinds of memory which memorise events in rank order of occurrence only, what our talent for music and speech shows is that in this brief storage we possess, with a working range of a second or so, a comprehension of those things we have just heard, arranged quite accurately in true time relationship.

This was explored by devising an electronic metronome in which every fourth beat was irregular by a controllable amount, the overall tempo or interval being also under control. It was set up connected to a loudspeaker in a studio with good listening conditions, and to a timer. The interval is first set to the required figure and the irregularity to zero, using the timer. The timer dial is then obscured, and the operator's task is to increase the irregularity himself until it becomes just evident to his ear. He may repeat until satisfied. Subdividing the intervals either overtly (by foot-tapping) or covertly (for which the operator's integrity must be relied on) is barred. This least discernible irregularity is then read from the timer.

A plot of two sequences of readings for various intervals obtained by the author after several weeks' practice is

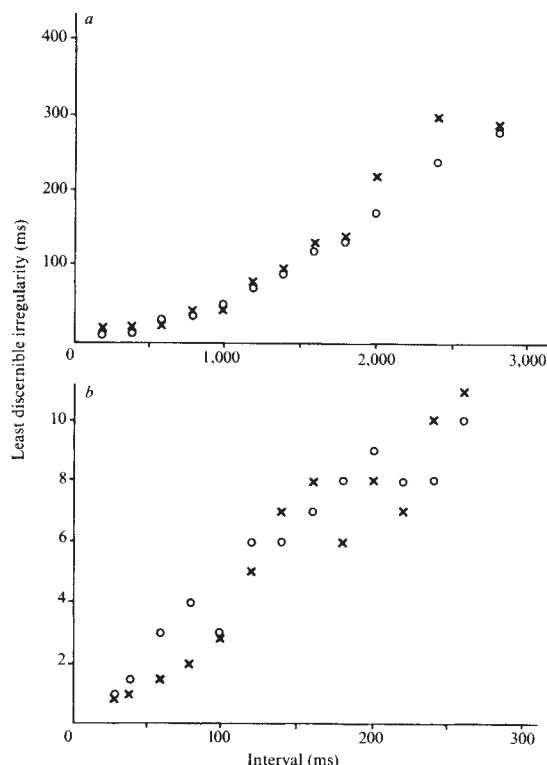


Fig. 1 The accuracy with which rhythm can be heard, or brief times assessed, plotted as least discernible deviation from regularity of a metronome, of which every fourth beat interval between beats. *a*, Intervals 260–3,200 ms; *b*, intervals 30–260 ms. Two runs are shown by the same operator on is controllably irregular, against overall tempo shown as different days. Considerable practice is needed to obtain results as consistent as these. ○, first run; ×, second run.

shown in Fig. 1. Similar plots obtained by other operators indicate that the limit of discrimination is biologically imposed. The experiment is difficult to perform but the effect of practice is to improve the consistency of results, not the discrimination under test.

The form of the plot is striking. There are no periodicities as might be expected from cyclic brain timing mechanisms, suggested by Lenneberg³. In fact the approximately exponential shape indicates that the brain mechanism involved in timing partakes of the nature of a simple analogue device, possibly of a decay time or delay line type.

I suggest that the above named arrangement of events in true time relationship occurs within this mechanism, displayed as it were against an exponentially decaying scale of the 3 s or so preceding the present instant; and that this, being man's only such arrangement, provides, in effect, our sensation of time.

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¹ Classe, A., *The Rhythm of English Prose* (Blackwell, Oxford, 1939).

² Neisser, U., *Cognitive Psychology*, 199–206 (Appleton Century Crofts, New York, 1967).

³ Lenneberg, E. H., *Biological Foundations of Language*, 107–120 (Wiley, New York, 1967).

matters arising

'Spatial oscillations' in the Zhabotinskii reaction

SIR,—In a recent study of temperature effects and the Zhabotinskii reaction, Thoenes¹ measured the speed of the reaction wave fronts and related it to the temperature distribution in the sample. A temperature gradient was artificially imposed from outside the system.

Thoenes' interpretation of the data is, however, either unclear or misleading on some points.

In the first paragraph Thoenes indicates that the theoretical analysis which he uses was suggested by Beck and Váradí². It seems more probable, however, that the reference should be to Zaikin and Zhabotinskii³. These authors refer to the work of Prigogine and Nicolis⁴, among others, as providing the theoretical foundation for their simplified analyses. Thoenes' implication that his theoretical analysis is independent of the work of Prigogine and coworkers is therefore misleading.

This analysis rests on the assumption that the frequency of the oscillations, ω , can be described as a function of the position, x :

$$\omega(x) = \omega^0 F(x) \quad (1)$$

This is Thoenes' equation (1). He later identifies $F(x)$ with

$$\exp(-E/RT(x)) \quad (2)$$

where E is an energy and $T(x)$ is the position dependent temperature. This is Thoenes' equation (5). Thoenes states that this treatment explains all the phenomena reported by Zaikin and Zhabotinskii³. This is not surprising as they had already shown this to be true. (In this context, recent work by Winfree⁵ is also interesting.)

But equation (1) can also be misleading in the context in which it is given. The oscillatory behaviour which it implies is rather rare in chemical reactions and the Zhabotinskii reaction is a very special case. Several authors have discussed the requirements for, and the characteristics of, such chemical reactions⁶⁻⁸. These works point out that equation (1) can only describe very special types of chemical reactions.

The interpretation of equation (2) may not be as simple as Thoenes suggests. The heat output and the redox potential step during the oscillations change according to the number of oscillations which have occurred⁹. Equation (2)

would not therefore be expected to give a single datum, as Thoenes apparently assumes. Some of the effects of heat production in the Zhabotinskii reaction have already been noted by Busse¹⁰, although his interpretations of some other points were incorrect¹¹. The chemistry of the Zhabotinskii reaction has been discussed extensively^{12,13}, and there has been a theoretical study¹⁴ of an ideal system of the type studied by Prigogine and coworkers but with heat production included. Beck and Váradí² also explicitly mention the effects of the container walls on the reaction characteristics.

Thoenes' statement "I conclude that spatial patterns in the Zhabotinskii reaction are not examples of dissipative structures as described by the theory of Prigogine" does not therefore seem to be correct. Thoenes' analysis is in fact based on Prigogine's work.

I conclude that Thoenes has reported very interesting experimental results on the effect of temperature on the Zhabotinskii reaction, and that in order to interpret this data he has also reformulated and enlarged upon a previous mathematical description by Zaikin and Zhabotinskii³ which was in turn based on the theories of Prigogine and coworkers⁴.

Yours faithfully,

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¹ Thoenes, D., *Nature phys. Sci.*, **243**, 18 (1973).

² Beck, M. T., and Váradí, Z. B., *Nature phys. Sci.*, **235**, 15 (1972).

³ Zaikin, A. N., and Zhabotinskii, A. M., *Nature*, **225**, 535 (1970).

⁴ Prigogine, I., and Nicolis, G., *J. chem. Phys.*, **46**, 3542 (1967).

⁵ Winfree, A. T., *Science*, **175**, 634 (1972).

⁶ Glansdorff, P., and Prigogine, I., *Thermodynamic Theory of Structure, Stability, and Fluctuations* (Wiley, London, 1971).

⁷ Prigogine, I., and Nicolis, G., *Q. Rev. Biophys.*, **4**, 107 (1971).

⁸ Prigogine, I., Nicolis, G., and Babloyantz, A., *Physics Today*, 23–28 November, (1972) 38–44 (December, 1972).

⁹ Körös, E., Orbán, M., and Nagy, Zs., *Nature phys. Sci.*, **242**, 30 (1973).

¹⁰ Busse, H. G., *J. phys. Chem.*, **73**, 750 (1969).

¹¹ Gray, B. F., *Nature*, **242**, 257 (1973).

¹² Field, R. J., and Noyes, R. M., *Nature*, **237**, 390 (1972).

¹³ Noyes, R. M., Field, R. J., and Körös, E., *J. Am. chem. Soc.*, **94**, 1394 (1972).

¹⁴ Rosen, G., *Phys. Lett.*, **43A**, 349 (1973).

Structure of water in porous glass

SIR,—In an attempt to measure proton mobility, Roberts and Northey, used signal line broadening from steady state nuclear magnetic resonance (NMR) to study slices of sintered glass with a pore size of $\sim 15 \mu\text{m}$ (ref. 1). They infer "long range modification of the water structure by sintered glass". Their conclusions do not necessarily indicate an ordering effect, and new evidence for the water-glass system casts doubt on their results^{2,3}.

Signal line broadening from steady state NMR has been used as an indicator of orientation effects for adsorbed systems. Interpretation of the data is, however, often ambiguous⁴. Pulse or spin echo techniques are considered more promising and have recently been applied to many systems⁵, including water on porous glass^{2,3}.

Our results, using pulse NMR, indicate that for a specially prepared porous powdered glass at 100% relative humidity, with an average pore size of $\sim 24 \text{ \AA}$, about 80% (v/v) of the adsorbed water is motionally restricted, and the remaining 20% (v/v) is most probably bulk water⁶. As the average pore radius approaches 100 $\text{ \AA}$, the amount of motionally restricted water in the Vycor glass is reduced to below 10% (v/v), which is approximately one layer of surface-bound water about 3 $\text{ \AA}$ thick (ref. 2). Thus, for very large average pore diameters, such as the 15 μm used by Roberts and Northey, with relatively small surface areas, it is probable that far less than 10% (v/v) of the occluded water is bound. In addition, because of the notoriously low sensitivity of NMR, detection of small percentages of bound water within very large percentages of bulk water is unlikely.

For the steady state NMR experiment, broadening which results from field inhomogeneities produced by the glass matrix would occur even if 100% (v/v) of the occluded water was free. Interpretation of the NMR data for adsorbed systems is far more complicated than the observation of the broadening effect⁶. For example, relaxation rates within, and exchange rates between, various environmental states (phases) critically affect the estimation

of the actual percentage of water within a particular state⁶.

These limitations seriously challenge the validity of Roberts and Northey's conclusions and probably account for the discrepancy between their results and those of Belfort *et al.*, who used the preferred pulse NMR techniques.

Yours faithfully,

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Jerusalem, Israel

¹ Roberts, N. K., and Northey, H. L. *Nature*, **237**, 144 (1972).

² Belfort, G., *J. Colloid Interface Sci.* (in the press).

³ Belfort, G., *Nature phys. Sci.*, **22**, 60 (1972).

⁴ Kaufman, S., Stein, J., and Gibbs, J. H., *Nature*, **225**, 743 (1970).

⁵ Pfeifer, H., *NMR, Basic Principles and Progress*, 5, (edit. by Dahl, P., Fleisch, E., and Kosfeld, R.) 53 (Springer Verlag, 1972).

⁶ Woessner, D. E., *J. chem. Phys.*, **35**, 41 (1961).

DR ROBERTS REPLIES: We acknowledge that Belfort's criticism might be valid had we simply recorded the NMR spectrum of water sorbed in the sintered glass. As pointed out in our article¹, however, the spectrum was recorded after distilled water had been drawn through the porous glass membrane for 30 h—the time normally required for the determination of a diffusion coefficient by the diaphragm cell method. The initial NMR signal of the water sorbed in the sintered glass was broadened compared with that of bulk water, and it might be argued that this broadening was simply a consequence of 'field inhomogeneities produced by the glass matrix.'

When water was drawn through the sintered glass for 30 h, however, the NMR signal of the occluded water became still broader, and an increase in hydrodynamic resistance also occurred (see also ref. 2). This increased broadening, which amounted to approximately 8 Hz at half-height, cannot be explained by invoking field inhomogeneities.

Belfort's work on porous glass³ does not discuss the effect of prolonged movement of water through glass pores. In this connection the observation² that the conductivity across a sintered glass disk immersed in dilute potassium chloride does not alter when the solution is drawn through the disk over an extended period, whereas the hydrodynamic resistance increases, suggests that the hydrodynamic resistance is not a result of obstruction of the glass pores by air bubbles or solid particles.

The purpose of our article¹ was not to argue that water in glass pores (5–15 μm diameter) is structurally modified. Rather, we argued that it becomes modified on prolonged movement through the pores, with a resultant decrease in the hydrogen ion diffusion (and most probably the diffusion of the hydroxide ion) but with no effect on the diffusion of other ions.

It would be interesting to be able to calculate the degree of broadening of the NMR signal of water which is compatible with a reduction in the value of the diffusion ratio, D/D° , of the hydrogen ion, from 1.0 to approximately 0.9. These were the values obtained respectively from the polarographic and the diaphragm cell methods.

Our recent work⁴ on deuterium ion mobility in heavy water solutions of electrolytes confirms our original finding that the diaphragm cell method gives low results for the limiting diffusion coefficient of the hydrogen ion.

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¹ Roberts, N. K., and Northey, H. L. *Nature*, **237**, 144 (1972).

² Mysels, K. J., and McBain, J. W., *J. Colloid Sci.*, **3**, 45 (1948).

³ Belfort, G., *Nature*, **237**, 60 (1972).

⁴ Roberts, N. K., and Northey, H. L., *J. chem. Soc., Faraday Trans. 1*, **70**, 253 (1974).

IQ and race

SIR,—In her recent communication Tizard¹ showed that the effects of environment on IQ are great but that racial differences are not significant. In fact, her data on children of 4.5 yr divided into those still in institutions, those returned to their natural mothers, and those adopted, show that the non-whites have a consistently higher mean score than the whites. The racial differences within each of the three environmental classes are, indeed, not significant but the probabilities may be combined, since the classes are independent, giving $P=0.04$. Taken alone, this result might not mean much, but in Tizard's other study non-white children have higher scores in each of three IQ tests than white children, the difference being significant for one test ($P=0.02$). It seems that the non-white children have higher IQs than the whites.

Unfortunately, this result is open to several interpretations, at least two of which seem probable. Non-whites may be better endowed genetically with regard to intelligence than are whites. On the other hand, however, the

children tested might not have been random samples of the racial groups to which they belong: British blacks are of recent immigrant stock and probably of above average socioeconomic status in their homelands. (Tizard found no significant differences between fathers of different races in ratio of manual to nonmanual occupations but this is a crude test and, in any case, blacks will do less well than whites of similar intelligence in job competition, because of colour prejudice and educational differences.)

The question of 'nature and nurture' with regard to IQ is politically sensitive: the extremists of both left and right are seeking support for equally silly and wicked beliefs. It is, therefore, important that the scientific examination of the question should be scrupulous. I hope that these comments will help the truth to emerge, whatever it may be and to whomever it may be unpalatable.

Yours faithfully,

J. J. D. GREENWOOD

Department of Biological Sciences,
University of Dundee

¹ Tizard, B., *Nature*, **247**, 316 (1974).

DR TIZARD REPLIES: Greenwood correctly states that the non-white children in our studies had consistently higher mean test scores than the white children. As I pointed out in my letter no definite conclusion can be drawn from this finding. Greenwood's suggestion that selective immigration by IQ has occurred from the West Indies is of course possible, but if true would constitute a powerful argument in favour of environmental influences on development, as it is known that the mean IQ of the school-age children of West Indian immigrants living with their families in London is significantly lower than that of white school children. An alternative 'genetic' explanation would be that West Indian immigrants who place their children in long-term care are of higher IQ than the corresponding group of white parents. 'Environmental' explanations of the finding could also be advanced; for example, small black children are often found particularly appealing and may receive more attention from their nurses.

The main argument of my letter, however, was that within each environment inter-racial differences in test scores were small, but differences in test scores which we could relate to measured aspects of the environment were large and significant.

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Is the slow worm a Batesian mimic?

SIR,—Smith¹ has suggested that juvenile slow worms (*Anguis fragilis* L.), and also those adult females which retain the dark vertebral stripe, are Batesian mimics of the adder (*Vipera berus* L.). His main evidence for this view is circumstantial; parturition occurs at the same time in the two species, but is usually about one week earlier in the viviparous lizard (*Lacerta vivipara* Jacquin). Smith suggests that parturition time in the slow worm has evolved such that the species gains maximum advantage from the mimicry.

There is, however, a simpler explanation of the time of parturition. The viviparous lizard is a strictly diurnal heliotherm, maintaining a 'preferred body temperature' of about 30° C whenever solar radiation is sufficiently intense to enable it to do so²; it can achieve this relatively high temperature rapidly³. Adders, in contrast, are both diurnal and crepuscular⁴, and the slow worm, as Smith admits, is a burrowing animal which is not often seen in daylight. Body temperatures of the adder are consequently variable; they do not maintain a high temperature for long periods. Slow worms do not usually maintain a preferred body temperature at all; their body temperature is nearly always near that of the ambient air (unpublished observations).

It is not surprising, therefore, that embryological development in viviparous lizards should proceed more rapidly, and it is significant in this connection that the optimum temperature for development of viviparous lizard embryos *in vitro* is 27–28° C, whereas for slow worms it is 18° C (ref. 5).

I conclude that there is no justification for regarding the slow worm as a Batesian mimic of the adder, and adduce as additional evidence the fact that neither my children nor I—two predators with very different levels of experience—ever confuse the two species in the field.

Yours faithfully,

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- ¹ Smith, R. H., *Nature*, **247**, 571 (1974).
- ² Avery, R. A., *J. anim. Ecol.*, **40**, 351 (1971).
- ³ Avery, R. A., and McArdle, B. H., *Br. J. Herpet.*, **5**, 363 (1973).
- ⁴ Smith, M. A., *The British Amphibians and Reptiles*, (Collins, London, 1951).
- ⁵ Maderson, P. F. A., and Bellairs, A. d'A., *Nature*, **195**, 401 (1962).

MR SMITH REPLIES:—Herpetologists have questioned my suggestion¹ that the dorsal zigzag of some female slow worms (*A. fragilis* L.) mimics that of adders (*V. berus* L.), since anyone familiar with the animals can easily distinguish them. Dr Bellairs (personal communication) notes the different behaviour of slow worms and adders when threatened, unlike other mimetic lizards², and Avery here suggests that the coincidence of parturition times is explained by body temperature and habits. The usual predators of slow worms are not humans, however, but are more likely to be small, colour-blind mammals which must decide quickly whether or not to attack. Thus a character associated with a previous unpleasant experience (an adder bite) may cause momentary hesitation, giving a slightly increased chance of escape and a small selective advantage.

Whether mimicry evolved in response to concurrent parturition times or *vice versa* is incidental to the increased advantage of a birth period around the peak of the adder's when gravid females are vulnerable to visual predation as they bask in the sun. Nevertheless, it is interesting that, although slow worms mate mainly in May, fertilisation does not occur until June³ and embryonic development begins around the birth time of viviparous lizards (*L. vivipara* Jacquin), so that 'preferred body temperature' is only part of the explanation.

Thus, Batesian mimicry seems to be the best, current explanation of the dorsal zigzag pattern and its presence in some adult females only.

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- ¹ Smith, R. H., *Nature*, **247**, 571 (1974).
- ² Bustard, H. R., *Br. J. Herpet.*, **4**, 22 (1968).
- ³ Smith, M. A., *The British Amphibians and Reptiles*, (Collins, London, 1951).

Silent alleles and haploid expression in deficiency mapping

SIR,—If a portion of one of the chromosomes is missing, and a primary gene product is reduced in amount, this indicates that either one locus determining this enzyme is missing or that it is ineffective due to a silent allele¹.

The evidence can be evaluated in the conventional currency used in human linkage studies, the lod, or logarithm of the odds ratio^{2,3}, by the following simple procedure.

We suppose that some transformation of the enzyme level is normally distributed with unit standard deviation, and is d units in the diploid state and h units in the haploid state: this may be derived from data on 'silent' alleles, on the assumption that they are completely silent, or 'non-leaky', or inferred by halving the diploid level. If the parent conveying this hypothetical silent allele has a level a units, after transformation, the likelihood that this is due to haploid expression is the ordinate of the normal curve of unit variance with mean h or $\sqrt{(1/2\pi)}\exp[-\frac{1}{2}(h-a)^2]$ while the likelihood that it is due to diploid expression is $\sqrt{(1/2\pi)}\exp[-\frac{1}{2}(d-a)^2]$; the natural logarithm of the ratio of these ordinates is $\frac{1}{2}[(d-a)^2 - (h-a)^2]$; and the logarithm of this ratio, or lod, is $0.217 [(d-a)^2 - (h-a)^2]$. The antilogarithm of this is the likelihood ratio or odds ratio.

On the data of Ferguson-Smith *et al.*¹ the mother had a mean level of 124 units; the mean level for controls with the same phenotype was 119 units and, taking the conservative estimate that a silent allele would reduce activity by 40%, we have $d' = 119$, $d = 15.4$; $h' = 71.4$, $h = 11.9$; $a' = 124$, $a = 15.7$ where the transformation $x = \sqrt{(2x')}$ is used. This gives an approximately unit standard deviation on the data of Spencer *et al.*⁴. The log of the likelihood ratio, or the lod, is then $0.217 [(a-h)^2 - (a-d)^2] = 3.1$ giving an odds ratio of about 1250:1 in favour of a nonsilent allele.

Supplementary information, such as the size of the deficiency, and the frequency of silent alleles, may also be incorporated. This is most simply done by working in logarithms throughout, so that the numerical results are additive.

The failure of the sister of the propositus to show evidence of a silent allele, which she would have a 50% chance of doing if one were there, provides a further lod of $\log(\frac{1}{2})$, or 0.30, favouring deficiency.

I thank Professor M. A. Ferguson-Smith for discussions.

Yours faithfully,

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- ¹ Ferguson-Smith, M. A., Newman, B. F., Ellis, P. M., and Thomson, D. M. G., *Nature new Biol.*, **243**, 271 (1973).
- ² Barnard, G. A., *J. R. Stat. Soc.*, **B11**, 115 (1949).
- ³ Morton, N. E., *Am. J. Human Genet.*, **7**, 277 (1955).
- ⁴ Spencer, N., Hopkinson, D. A., and Harris, H., *Nature*, **201**, 299 (1964).

Muscle cross-reinnervation

SIR,—In a recent communication in *Nature*, Weeds *et al.*¹ described some modifications in the subunit constituents of myosin subsequent to surgical transposition of the nerves of two functionally disparate muscles (for example, a fast-twitch, glycolytic muscle against a slow-twitch, oxidative muscle). The evident metabolic reprogramming of the cross-reinnervated muscles led them to conclude that "It would be interesting to know if the electromyogram activity of muscles does change following cross-reinnervation" (that is, is the alien-innervated muscle activated in a fashion comparable with that of the muscle for which the nerve was normally intended?).

Obviously, few if any efforts have been made to determine the actual pattern of utilisation of cross (and self) reinnervated limb muscles. Ideally such studies require precision monitoring of electromyogram activity (by chronic indwelling electrodes) or normal and alien-innervated muscles during the repertoire of physical activities of normal and experimental animals. There are, however, several less sophisticated observations available which indicate that cross-reinnervation by functionally dissimilar nerves does lead, at least partially, to the expected changes in patterns of activity of the affected muscles. Furthermore, an exchange of functionally similar nerves apparently allows some retention of the original manner in which a muscle was utilised.

In the course of several investigations on rats, I surgically rerouted either the phrenic nerve, or hypoglossal nerve, to a denervated ipsilateral sternomastoid muscle. Several months thereafter the animals were lightly anaesthetised and the function of the sternomastoid muscle was observed directly through an incision in the neck. After phrenic reinnervation the sternomastoid muscle contracted rhythmically in concert with the intact contralateral hemidiaphragm (an activity that could be enhanced by temporarily occluding the air passages). Following hypoglossal reinnervation the sternomastoid twitched intermittently in conjunction with licking motions of the tongue. The extent to which this transposition of functional activity from diaphragm to sternomastoid, or tongue to sternomastoid is operative in the fully awake animal is uncertain. Nevertheless, the appropriate circuitry and functional capacity of the phrenic and hypoglossal motorpools apparently can be diverted through a cross-nerve anastomosis to a muscle (the sternomastoid) that is not primarily responsible for breathing or licking activities.

The functional consequences of

self-reinnervation, rather than cross-reinnervation, have also been answered in part by Guth and coworkers² who demonstrated in the rat that the recurrent laryngeal nerve, some fibres of which normally transmit efferent bursts synchronous with those of the phrenic nerve, is often capable of restoring rhythmic diaphragmatic activity subsequent to a recurrent laryngeal-phrenic nerve anastomosis.

Sperry³, in still earlier studies, emphasised that a regenerated nerve tends to retain its original reflex activity.

Thus, in spite of the disparity in muscle nerves (with respect to quality and quantity of efferents and afferents, and the differences in central circuitry) there is good reason to assume that many of the nerves used in cross-reinnervation experiments continue to elicit a gross pattern of muscle activity quite similar to that of their original musculature. It is difficult, however, to envisage a precise replication of function in most cross-reinnervated muscles because of the extreme complexity and uniqueness of each nerve, each muscle, and the myriad factors interwoven in the nerve regeneration process.

Yours faithfully,

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Diseases and Stroke,
United States Public Health Service,
Department of Health, Education, and
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National Institutes of Health,
Bethesda, Maryland 20014*

¹ Weeds, A. G., Trentham, D. R., Kean, C. J. C., and Buller, A. J., *Nature*, **247**, 135 (1974).

² Guth, L., Soutter, L., Frank, K., Campbell, J. B., and Lloyd, J. B., *Expl Neurol.*, **2**, 251 (1960).

³ Sperry, R. W., *Q. Rev. Biol.*, **20**, 311 (1945).

THE AUTHORS REPLY:—Dr Yellin's observations are certainly pertinent to the question posed by us, and are also in line with expectation following the pioneering work of Sperry² to which Dr Yellin refers. The broader problem to which our query relates, however, is whether the altered patterns of motor nerve impulses which probably reach both fast-twitch and slow-twitch mammalian skeletal muscles following cross-reinnervation are by themselves sufficient to bring about the mechanical and biochemical changes which are now known to follow altered innervation. For this to be so at least four conditions must be met.

First, it must be established that alterations in the number, and or, pattern of nerve impulses reaching a normal muscle can alter that muscle's mechanical and biochemical character-

istics.

Second, it must be demonstrated that following cross-reinnervation, there is an altered pattern of motor activity in the reinnervated muscles.

Third, it must be shown that the change in activity pattern following cross-reinnervation is quantitatively correct to account for the observed mechanical and biochemical changes.

Fourth, it is also necessary to demonstrate that the observed changes in the mechanical and biochemical characteristics of cross-reinnervated muscle are the direct result of the number, and or, pattern of motor nerve impulses, and are not due to some concomitant metabolic change occurring in the motoneurone as a consequence of its altered activity.

The first point is now established beyond doubt by the original experiments of Salmons and Vrbová³, and the later work of Streter *et al.*⁴ and Al-Amood *et al.*⁵. Dr Yellin's observations add weight to the expectation that the electromyographic activity of both fast-twitch and slow-twitch muscle is altered by cross-reinnervation, but, as he implies in his letter, the satisfying of the third requirement must await the quantification of the appropriate electromyograms from conscious, unrestrained, cross-reinnervated animals.

At the present stage of knowledge, consideration of the fourth requirement can only be speculative^{5,6} but it is clear that if the changes following the cross-reinnervation of mammalian fast-twitch and slow-twitch muscles are to prove entirely explicable in terms of the pattern of motor nerve impulses reaching the muscles, all four criteria listed above must be satisfied. Only then can the additional possibility of another neural (trophic) factor operating in this situation be firmly rejected.

Yours faithfully,

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book reviews

Mathematics of the Universe

The Large Scale Structure of Space-Time. By S. W. Hawking and G. F. R. Ellis. Pp. xi+391. (Cambridge University: London, May 1973.) £10; \$28.50.

THERE has been a considerable upsurge of interest in general relativity theory over the past ten years or so. This is undoubtedly partly due to several remarkable discoveries in observational astronomy in recent years (quasars, pulsars, the microwave background, explosions in galactic centres, and so on) which indicate the presence of gravitational fields in nature so strong that Newtonian theory seems unlikely to be completely adequate. But equally important to Einstein's theory, and largely independent of such observational considerations, have been a number of mathematical developments in the theory. The work of Bondi, Sachs and others on the positivity of energy carried by gravitational waves was an important landmark. So was the development of techniques for finding exact solutions of Einstein's field equations, leading to the discovery by Kerr of a solution representing the field of a rotating isolated source. More recently, topological methods have been developed and have proved to be increasingly valuable for the treatment of problems where an exact analysis is not yet feasible. It has been possible, in particular, to conclude that the presence of singularities in solutions of Einstein's equations is not just a feature of the high symmetry that had been normally assumed. It is now known, for example, that irrespective of symmetry the collapse of a massive star will, according to Einstein's theory (once a point of no return has been reached), result in a space-time singularity where it is to be expected that densities and curvatures will diverge to infinity. A similar singularity occurs in the initial stage of the expansion of the Universe—again irrespective of symmetry. In the case of a collapse, the resulting black hole can be studied by a variety of techniques, with topological and analytical arguments each enhancing the value of the other. These results have been the work of several different theorists, the name of S. W. Hawking being pre-eminent among them.

Until recently, no complete account

of these later topological and related developments has been available, but the situation is now happily remedied with the publication of this book. It is an impressive *tour de force*. It starts with a detailed but rapid introduction to differential geometry; this is followed by a discussion of the physical basis of Einstein's equations; then there is an elaborate discussion of conjugate points on geodesics; next, many of the most important exact solutions of Einstein's field equations are studied in detail, including Kerr's solution, the frequent use of space-time diagrams being a most welcome feature; causal properties of space-times are then discussed and several useful theorems are proved; asymptotically simple space-times are analysed; then there is a detailed discussion of the Cauchy problem; the singularity theorems are next described and proved in detail; the Schmidt *b*-boundary is introduced to give a precise definition of a singularity; then the black hole theorems are discussed and proved; finally there is an analysis of the status (derived partly from observations of the microwave background) of the initial big bang singularity. Much of the later material is presented here for the first time; the book also is a remarkably complete survey of the relevant material. This book is clearly a 'must' for any advanced student who wishes to follow up these modern developments in any detail.

But he will have to work hard. The book is not, in my estimation, at all an easy one to study in detail. Much useful information can, no doubt, be obtained by browsing and the flavour of the arguments can be ascertained. But for a detailed appreciation of the techniques, there is a number of serious obstacles for the student. Some of these are intrinsic to the subject. The very breadth of knowledge involved, ranging from pure mathematics to observational astronomy, is in itself a serious hurdle. Most of the book is mathematical in nature. But it is relevant mathematics. Little concession is made to mathematical elegance for its own sake and this can make for difficulties in reading. I had the feeling also that in many places the arguments could have been simplified and greatly clarified by a certain amount of reorganisation. But it is perhaps unfair to ask of the authors that

they should have produced a polished gem of clarity for work which is so new. One knows only too well the enormous extra labour that can be involved when one attempts to reduce a presentation to its best possible form.

Nevertheless, I feel that certain points of criticism may reasonably be made. There are several small errors and ambiguities present which can make reading difficult. (I noticed something like sixty of these but I found no serious errors. It should be pointed out that with the association of tensors with forms that is quite reasonably made, a factor $n!$ should occur with the definition of the integral of an n form; relation (3) on page 32 is wrong; proposition 4.5.12 seems not to be true as stated; some of the diagrams suffer from being inaccurately drawn, such as in Fig. 31, the "matter world-line" seems to be spacelike.) Also, key definitions are often given in the middle of paragraphs and can come upon one unexpectedly (for example on page 195 a local causality neighbourhood is defined in parenthesis). Perhaps this need not be a drawback if done systematically, but the concept of "Cauchy development" is essentially defined three times, on page 60, page 119 and page 201.

I should have been happier if the physical significance of the metric g had been made more clear cut, defined by the behaviour of clocks as emphasised by Synge and Bondi. I feel it is a pity that the causality and chronology symbols $<$, $\ll$ are not used, since they can greatly simplify the presentation of some arguments. The terminology 'closed trapped surface' is used frequently in the book, but I found no mention of a 'trapped surface'. A reversion to this latter (original) terminology would seem a worthwhile simplification. Whereas indexed symbols are used to denote sets of components rather than actual tensors, there are places where the notation seems, rather confusingly, to indicate the contrary. In the discussion of the Cauchy problem in chapter 7 there are many very detailed formulae but little explanation of the ideas involved. I found this very hard to follow. So also were a number of other proofs. The black hole collision depicted in Fig. 60, and in particular the behaviour of the apparent horizons, is not supported in the text by any formal statements. The conjectural aspects of this picture should have been brought out. I feel that credits are

sometimes given rather haphazardly (for example there is no mention of Newman in connection with the charged Kerr solution, nor of Bondi in relation to the mass carried by gravitational waves). Finally, the absence of any discussion of 'mixmaster' singularities or of any details of the Belinskii - Lifshitz - Khalatnikov work seems to me to be a serious omission.

I would like to emphasise that these criticisms are basically minor ones in view of the formidable and impressive task undertaken by the authors. They have succeeded admirably and their work is undoubtedly a landmark in the presentation of modern gravitational theory.

The question of where the universe comes from and where it is going cannot fail to be of interest to everyone. There is rather little in science which directly bears on this question, but if anything seems to, it is surely the subject matter so powerfully presented in this book.

ROGER PENROSE

Food manufacture

Biology and the Food Industry. By H. R. Barnell. Pp. 59. (The Institute of Biology's Studies in Biology, No. 45.) (Arnold: London, February 1974.) £1.50 boards; 75p paper.

THE story is told of an Oxford undergraduate who obtained consistently good grades for his essays by copying from the *Encyclopedia Britannica* which his tutor had never read. To help "students at school, college and university . . . to keep abreast of recent trends and know where the most significant developments are taking place", the Institute of Biology sponsors the preparation of a series of booklets on selected aspects of biology to supplement the more substantial but inevitably finite treatment of biology which a textbook could be expected to provide. The late Dr Rex Barnell has written such a booklet about the food industry. In it he has systematically described such salient features of manufacturing processes and methods of food preservation as could be expected within the constraint of 59 pages, bearing in mind that he had also to touch on food composition, nutrition and the future. He has performed his task well and both pupils and their teachers will be grateful whether or not there may be some readers who feel a sneaking sympathy for the Oxford tutor.

Here will be found the distinction between drying and dehydration, a definition of a convenience food, description of pasteurisation, canning, chilling, freezing, irradiation and four lines

about pickling. Ten pages suffice for milling, baking and margarine making. Vegetable meats, leaf protein and micro organisms grown on hydrocarbons get seven pages. It is all very neatly done. The section devoted to nutrition and food quality sensibly draws attention to the contribution manufacturers make to an adequate diet by making their commodities readily available. In the breathless pace, however, figures for energy value seem to have slipped out of the table of "recommended intakes". The book ends on a high note with the promise that "it is by no means impossible that if groups of biological, physical and chemical research workers concentrate on the problems . . . virtually unlimited supplies of food . . . may be truly synthesised . . . from constituents of the earth's atmosphere and crust". Well, perhaps they may.

MAGNUS PYKE

Reactions at electrodes

The Chemistry of Electrode Processes. By Ilana Fried. Pp. x+225. (Academic: London and New York, January, 1974.) £4.90; \$13.75.

RECENTLY, a number of books have appeared based on the study of electrode reactions or electrodictics. According to the author, this monograph is designed as "an introduction to electrodictics" for "the advanced student or the qualified chemist" who possesses "no familiarity with electrochemistry". The author has succeeded in achieving her goal and has produced a concise and very readable book. Every section is prefaced by a clear statement of the question to be answered and derivations have been kept simple by mixing rigorous treatments with comparisons and analogies. The SI unit system and IUPAC conventions have been adhered to throughout. Furthermore, the book contains a balance between theory, experimental details and areas of application.

The book is divided into six chapters; the first two are brief and introduce the area of interest, the fundamental concepts and definitions. The third chapter deals with the kinetics of electrode reactions by developing the relationships between current, potential and time for both reversible and irreversible electron transfer processes. Moreover, irreversible processes caused by slow chemical reactions preceding charge transfer and reactant adsorption are also considered. Finally, the influence of cell impedance on the current-potential behaviour is discussed. The fourth chapter which completes the theoretical part of the monograph is devoted to the electrode-solution interphase. Various models of the double layer are evalu-

ated and the modern model which offers the best fit to the experimental results is described. The topic of specific adsorption is also examined because of its effects on the double layer. The final section discusses how electrode reactions are influenced by the electrode-solution interphase.

The fifth chapter introduces the methodology of electrode reactions. Included in this section are descriptions of cells, electrodes and apparatus; equilibrium measurements of capacitance and impedance; voltammetric methods; and potential, current and charge step techniques together with their limits of applicability. The chapter concludes by mentioning the techniques of reflection spectroscopy and transmission spectroscopy at optically transparent electrodes. One drawback to this chapter, in particular, is the lack of references to the literature. In fact, the bibliography contains only a selection of books that the author "found useful" and omits all references to recent reviews and original contributions.

The final chapter offers little more than an indication of the number of areas where electrode reactions are involved, for example, corrosion, electroplating, and fuel cells. This broad survey was too ambitious and the author would have been more successful in illustrating the applications of electrodictics by examining, in detail, a limited number of solutions to problems related to the areas mentioned. Such an approach would have supplemented the material of chapter 5 and, by involving the reader in the problem-solving process, would have further familiarised him with the study of electrode processes.

C. M. ELSON

Cultured plants

Plant Tissue and Cell Culture. Edited by H. E. Street. Pp. viii+503. (Botanical Monographs, Vol. 11.) (Blackwell Scientific: Oxford and London, 1973.) £12.50.

ALTHOUGH plant tissues were first cultured *in vitro* 35 years ago, the realisation of their potential has always seemed to remain tantalisingly in the future. The technical problems of initiating and maintaining cultures of all but a few particularly favourable plants have until quite recently seemed insuperable obstacles to achieving the ready answers to physiological questions that the tissue and cell culture approach seems to offer. This excellent book should completely dispel that notion. Professor Street has a knowledge and experience of the culture of plant tissues, and particularly plant cells, that is second to none, and with his cust-

omary energy and enthusiasm has produced a superb account of the technicalities and potentialities of treating plants as microorganisms. He has been ably assisted in this by first-class contributions from a group of experts in specific aspects of the topic, although the editor's organising and integrating influence can be discerned throughout the book.

Approximately half of the book is devoted to a careful and comprehensive description of the many different techniques available for the culture of plant tissues and cells, beginning with an extremely useful account of the basic essentials of a tissue culture laboratory written by the editor himself. Street also provides a chapter on the apparatus and methods necessary for the successful cultivation of plant cells as suspension cultures. This includes full descriptions of batch culture chambers and of automatic chemostat and turbidostat devices. The technicalities of initiating and maintaining callus cultures are admirably covered by M. M. Yeoman, from Edinburgh, whilst E. C. Cocking and P. K. Evans of Nottingham discuss the isolation, culture and potential of plant protoplasts. The exciting topic of the production of haploid cell lines, with their obvious implications for plant genetics, receives a scholarly review by N. Sunderland, from the John Innes Institute, in a chapter dealing with pollen and anther culture. The general cytology of cultured cells is described in a long, informative chapter jointly written by Yeoman and Street, and Sunderland separately discusses the vexing question of the karyological instability of plant cultures.

The growth pattern of callus cultures and cell suspension cultures is treated in depth in separate chapters by Yeoman and P. A. Aitchison, and by P. J. King and Street. The discussion on cell suspension cultures is particularly valuable since it not only provides information on the growth and physiology of isolated plant cells, but also shows in great detail how highly synchronous populations of cells may be established. Such cultures can exhibit a remarkable synchrony of cell division for up to eight generations and, with a cell cycle of around three days long, their potential for the intensive investigation of the control mechanisms of the cell cycle must surely be greater even than that of the yeasts and unicellular algae. The book is rounded off by discussions of organogenesis and embryogenesis (J. Reinert, Berlin), the culture of plant tumour cells with special reference to crown gall (J. N. Butcher, Cambridge), and the growth of plant parasites in culture (D. S. Ingram, Cambridge).

Plant tissue culture is no longer an end in itself; it has progressed to a point at which it can be considered to be a

highly sophisticated technique capable of providing answers to problems throughout the whole of plant biochemistry, physiology, development, genetics and pathology. This beautifully produced book will be invaluable to the rapidly growing number of plant scientists who wish to use plant tissue or cell culture, but who do not wish to devote their time to becoming experts.

HARRY SMITH

Big game ecology

Wildlife Ecology. By A. N. Moen. Pp. xvi + 458. (Freeman: San Francisco; 1973.) \$17.50.

SYSTEMS analysis and predictive model building in ecology are at an interesting stage in their development. More and more autecological studies pour out increasing quantities of details for synecologists to work on. This book is a synecological study of north American big game. It is unique in that it attempts to describe the physical and biotic interactions of game with their environment and to assess the importance of these interactions in terms of overall productivity. Their influence on game and range management are clearly stated. The title of the book is misleading, for only a small amount of space is devoted to wild fowl, gallinaceous birds and other wild life. Most of the data and examples are taken from studies on elk (wapiti), mule and white tailed deer and caribou.

The chief value of this book is that it brings between two covers a multitude of details regarding methods of measuring environmental parameters. Working ecologists and game managers need to know these details, but cannot afford much time ferreting them out. They will find this aspect of the book most appealing. Particularly useful in this respect are the two chapters which deal with the effects of climatic conditions on physical characteristics of the environment and on large ungulates. The functional relationships between weather and organisms are minutely analysed from the viewpoint of transference of thermal energy. The distribution of thermal energy is the commonest bond in these relationships and from this is drawn a conceptual picture of the complex nature of heat exchange involving all the basic modes of heat transfer. Anyone actively involved in game management, as well as theoretical modellers, will find this section of the book of immense value.

Physiological and behavioural interactions between game and environment are covered in the first half of the book in thorough, but not long winded, detail. The second half deals with the analysis of interactions involving both

these biotic interactions and the physical ones. Emphasis is laid on that essential statistic for range managers, the carrying capacity of the habitat. Effective calculation of this is possible only by computer simulation and integration of models of all aspects of the system being analysed. The author suggests a useful working format that such models should follow.

This book has, to my mind, two outstanding merits. The first is that it paints a detailed picture of the ecology of northern big game, in the style of Canaletto rather than that of the Impressionists. This alone should commend it to the attention of pure and applied ecologists alike. The second is that the book inspires the reader to think big; in terms of systems rather than species. For the development of ecology this seems like a good thing and for successful environmental manipulation an essential thing.

Good game management, like all ecosystem management, demands that decisions be taken on ecological bases. The benefit of step by step model building means that decisions taken can, before implementation, be tested by computer simulation. The degree of similarity between computer prediction and observed dynamic change depends on the realism of the biological models involved in the computation. No one must forget that this is the fundamental core of ecology which always has and always will depend on the skills of the individual field ecologist. As far as north American big game are concerned, this book brings us one step nearer rational management.

D. MICHAEL STODDART

Rho-sigma relations

The Hammett Equation. By C. D. Johnson. Pp. viii + 196. (Cambridge University: London, October 1973). £4.50; \$13.50.

TO L. P. Hammett is owed the recognition that polar effects of aromatic substituent groups on rate and equilibrium constants of side-chain reactions are quantitatively regular. This conclusion is formally expressed by Hammett's $\rho\sigma$ relation, to which the volume under review is devoted.

The author provides an elementary, yet fairly detailed account of the role of Hammett's treatment in organic chemistry. It is intended for senior undergraduate or first-year graduate students, a formidable aim since, even for the simplest cases, general statements have to be tempered by ifs and buts. The book describes the various developments of the original $\rho\sigma$ treatment and provides a guide to the brood of modified sub-

stituent constants (for example σ^+ , σ^- , σ^* , σ° , σ_I , σ_D , σ_R , σ^n , σ^G) which, on the one hand, extend its practical usefulness and, on the other hand, rob it of its beguiling simplicity and blur its theoretical significance.

It can be argued that the two chief motive forces for the development of science are the establishment of unifying principles (which provide the landmarks in the history of science) and the destruction of these same unifying principles by the experimental falsification of their predictions. Although Dr Johnson would hardly present the issue in those terms, the Hammett equation provides an interesting illustration of the interplay of these forces in the current development of chemistry.

V. GOLD

Quantum mechanics

Principles of Quantum Mechanics: The Non-Relativistic Theory with some Illustrative Applications. By W. V. Houston and G. C. Phillips. Pp. xi+376. (North-Holland: Amsterdam, 1973.) Dfl.110; \$44.

THIS book covers the standard undergraduate course in quantum mechanics. In the introduction it is suggested that students should already have met some of the physical ideas but in fact the book is self contained and would be a suitable textbook for a full year's course for students who have no previous knowledge of the subject. In a field which is already reasonably well populated with good textbooks this volume will not be out of place. It cannot, however, claim to have any significant new features.

It is hard to see how the chapter on classical mechanics fits into the general scheme of the book. Although it is sometimes claimed that Poisson brackets and the Hamilton-Jacobi equation are an aid to an understanding of quantum mechanics these topics do not appear to be used at all in the presentation of quantum mechanics given in this book so their presence in chapter 2 is an unnecessary hurdle for the student. Another slightly unsatisfactory feature is that a reader might be left with the impression that there are two rival 'quantum theories' which happen to give similar results, namely wave mechanics and formal quantum theory. Of course it is clearly stated that these are equivalent but the method of presentation, even involving two separate sets of axioms, is not conducive to making this obvious. The notation used in chapter 8 does not always help.

Several sections of the book, for example the chapter on scattering theory, do not make easy reading but this is

partially due to the fact that more care is taken in presentation than in some similar books.

The many interesting examples in the book will be found useful, as will the brief mathematical appendix.

E. J. SQUIRES

Condensed matter

The Structure and Properties of Solids: The Crystal Structures of Solids. By P. J. Brown and J. B. Forsyth. Pp. 172. (Arnold: London, July 1973.) Boards £4.40; paper £2.20.

THIS book aims to give an understanding, at undergraduate level, of the important elements in the study of the crystal structure of solids. There are chapters on crystallographic geometry and symmetry, the production and properties of X rays, neutrons and electrons, and the theory of diffraction, including the scattering of the different radiations. The experimental study of diffraction is described, and is followed by three chapters describing, and to some extent rationalising, the structures of the elements, ionic compounds and binary alloys.

To attempt to describe all this body of work inside 172 pages is itself a major achievement, and the authors certainly have given the reader a large amount of information at a relatively low price. The book also contains much useful practical information not usually found in physics textbooks. For example, the use of the ASTM index for identifying powder patterns is described in detail, and the 'cross-multiplication' rule for calculating the zone indices of the intersection of two planes is given. Both of these aids are used frequently by crystallographers in practice, and it is good to see them (and many more) in a book of this kind.

It is perhaps not surprising that the authors have had some difficulty in coping adequately with the wide range of subjects in such a slim volume. For the book is really a distillation of three types of book: one on crystal symmetry, like Phillips' *Introduction to Crystallography*; one on diffraction theory and techniques; and one like Evans' *Crystal Chemistry*. Though it would be nice to think that an undergraduate could master all this information quickly because it is presented in a concise way, I must say I am sceptical. Much of the writing is almost in note form, and the level of explanation is often such that I can only understand the point made, because I, like the authors, have studied the subject before in a more leisurely fashion.

The list of topics to leave out is clearly the key decision in a book of this length, and although no two authors

would agree on the list, the choice seems to have been exercised somewhat unevenly. Rather complex examples of non-crystallographic point groups and the idea of magnetic symmetry are introduced in chapter 1, and the complete derivation of the formula for the integrated diffracted intensity is in chapter 3. But space has not been found in chapter 5 for a description of how crystal structures are experimentally determined; after the formal theory, the reader could usefully be rewarded with at least a simple example of a structure determination.

Apart from this inexplicable omission, the book is a very good summary of the various aspects of the study of crystal structures. As such it will be useful for research workers who wish to survey the field quickly. It could also be usefully used as an undergraduate text; however, thought should be given to the speed at which the topics can be mastered, as there is as much material here as in most books of three times the length.

J. A. VENABLES

Dirty air

Fundamentals of Air Pollution. By A. Stern, H. C. Wohlers, R. X. Boubel and W. P. Lowry. Pp. xiv+492. (Academic: New York and London, September 1973.) \$14.50.

THIS book represents a welcome addition to the literature on air pollution and will serve primarily as an introductory book to the field. It is divided into four parts respectively on the elements, effects, meteorology and control of air pollution. The authors intended to introduce the topic in part 1, which together with part 2 defines the problem, and to resolve it in parts 3 and 4. These objectives are not completely met and the book gives the impression of being four short books condensed into one. It is difficult for instance to understand why the sections dealing with sampling and analysis (chapters 14 and 15) have been included under "The Effects of Air Pollution" while the section dealing with isokinetic sampling techniques occurs in part 4.

Much of the analytical work is presented very poorly with undue emphasis on classical procedures. In contrast the meteorology section is well presented and informative. The section which must receive the greatest criticism is that on the control of air pollution. Here the authors go back to consider sources of air pollution and sampling techniques, devoting only twenty pages to the engineering control of emissions from both vehicles and industrial processes. This, in a book of 450 pages, is surely inadequate.

R. PERRY